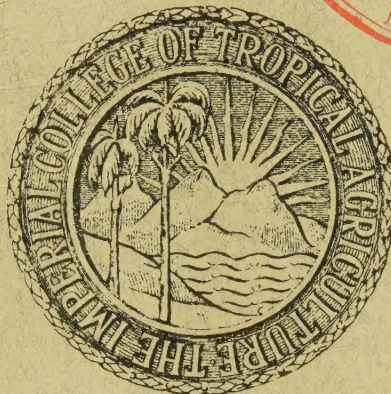
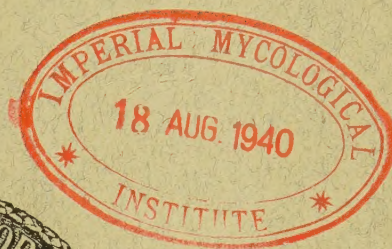


NINTH ANNUAL REPORT ON CACAO RESEARCH 1939



Via colendi haud facilis.

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NINTH ANNUAL REPORT ON CACAO RESEARCH, 1939.

A.—BOTANICAL SECTION.

THE BOTANICAL PROGRAMME OF 1939.

By E. E. CHEESMAN.

IN recent Reports the main plan of our research programme has been somewhat in the background, because the planting of field experiments, and the routine propagation necessary to provide material for them, do not provide much to write about. Such work, however, goes on steadily and there are now nearly six acres (1,728 trees) planted at River Estate in the main experiment comparing selected clones and propagation methods. A further 1,152 trees are being prepared for planting in 1940. Among the 576 trees of the first set of randomised blocks, planted in 1937, several bore one or two pods this year, and records will be opened on this set in the 1940-41 season.

The trees which bore were mostly I.C.S. 1, which has now shown on several sites that as a clone it is a notably early bearer, though several others show a similar tendency. There are strong indications of important differences between clones in this respect.

In conjunction with the routine propagation, still carried on by Mr. Spencer, records have been kept which enable us to estimate costs, and a note on this subject is included in the report.

Whilst we wait for the new clonal material to mature, Mr. Cope has continued his studies of self-incompatibility and other factors involved in analysis of fruitfulness. The detailed observations on 600 young seedling trees at River Estate, which have already given interesting information, were kept up for another season, and gave results broadly corroborating those of last year. Once again the self-compatible trees flowered less than the self-incompatibles but set a higher proportion of cherelles, and after losing more by shrivelling finished up with a heavier crop.

Although the trees were a year older, they bore in the aggregate almost exactly the same crop as in the year before. It appears that the

increase which might have been expected as a consequence of age was balanced by a reduction due to an unfavourable season. Thus increased flowering and setting were offset by increased shrivelling. In these circumstances it is interesting to notice that although the final effect of season on yield was the same for both classes of trees, the effects on intermediate stages were slightly different. The *percentage* of flowers setting was lower on the compatibles and higher on the incompatibles than in the year before. This suggests that the second season was possibly less favourable to the agents of self-pollination and more favourable to the agents of cross-pollination than the first.

The suggestion draws attention again to the fact that analysis of factors contributing to yield cannot be completed until we can close the gap in our knowledge of pollinating agents. As a step in that direction, an additional observation was made on some of the same young trees over a period of 14 months, namely, a fortnightly record of insects found in the flowers. The study disclosed an interesting statistical correlation between setting and the density of both red ants and thrips in the flowers. Mr. Cope rightly emphasises that this result is not to be hastily interpreted as proof that these insects are agents of pollination. It is, however, evidence on which they may reasonably be brought to trial, and the observations should be useful to the entomologists who are helping us in the search for the pollinating agents of cacao.

Further studies of the mechanism of self-incompatibility have confirmed the finding that it is not, in cacao, a matter of differential rates of growth of pollen tubes. Mr. Cope's careful comparison of changes in the embryo-sac after compatible and incompatible matings will be of more interest to geneticists than to cacao planters but they have an important though indirect bearing on our studies of fruitfulness.

THE COST OF CACAO PROPAGATION.

By E. E. CHEESMAN AND G. E. L. SPENCER.

THE propagation of cacao by cuttings, whilst still regarded as an experimental process, has now passed from one phase of research to another. The technique is established, and young trees raised from cuttings are in bearing.

Their performance, so far, is very promising. There are still only a few of them old enough to give useful records, since the first large planting at River Estate was rather late in 1937. There are, however, 51 three year old trees on San Juan Estate which have been recorded through the 1939-40 crop by the College Department of Economics. Of these, 41 are in bearing, and gave during the year 68.6 lb. of cacao, an average of 1.3 lb. per picket. The 51 trees belong to 11 different clones—some represented by only a single tree—and some of them seem to be relatively slow in coming into bearing. The figures for the four clones making up the bulk of this planting are:—

Clone.	Pickets.	Bearing trees.	Total yield. (lb.)	Average per picket.
I.C.S. 1	15	13	30.2	2.0
4	8	6	12.4	1.5
22	8	7	5.9	0.7
45	6	5	9.6	1.6
Total ...	37	31	58.1	1.6

These trees are growing in a replanted field on chocolate soil, which is one of the best cacao soils in Trinidad, and they have had every care from the time of planting. Similar yields are not to be expected from "cuttings" planted on the poorer soils or neglected after planting; nor is the bearing likely to be quite so early where the trees are used as "supplies". With these reservations, the figures still show that the new method has potentialities, and is well worth further study.

In the translation of any new technique into practical agricultural or horticultural procedure, there is inevitably a rather long "experimental" period during which a careful watch must be kept for the development of unsuspected defects or disadvantages. In this period the practical agriculturist or horticulturist, rather than the agricultural scientist, must be the chief experimenter. It is necessary that the new technique shall be tried out under a variety of commercial conditions, and on a scale large enough to be profitable if it succeeds, yet not so large as to be ruinous if it should happen to fail. The scientist may advise and help, but commercial growers must carry out the work for themselves before they can decide whether it really is profitable.

Thus for more than a decade budded rubber was tested on a commercial scale in the East, cautiously, but with gradually increasing confidence. We may hope that the history of vegetatively propagated cacao in the next decade will be similar.

Adequate testing will involve the planting of many thousands of vegetatively propagated trees, and it becomes important to estimate the cost of rooting cuttings on a commercial scale.

Our own experiences with the I.C.T.A. propagating plant have not been exactly on a commercial scale, but in the six months from 1st July to 31st December, 1939 we kept records of output and labour requirements from which a rough estimate can be calculated. In this period there was enough material to keep the equipment working at full capacity, and after culling out weaklings we took from it 1,870 good plantable young trees. This was "wet season" propagation, and there is usually some drop in the drier half of the year, but 1939 was a year of low rainfall and the rooting percentage was not up to the usual level. The capacity of the plant in a full normal year may therefore be placed conservatively at 3,600, or 1,200 from each of the three "batteries" of propagating bins in use. In a favourable year, or in a district more suitable for cacao nurseries than St. Augustine, the output might easily reach 1,500 per battery, but our calculations are based on the 1,200.

Our observations on labour suggest that if we had not much other horticultural work proceeding at the same time our plant would be too small for economical working. A more economical and convenient unit would be one of twice the capacity, and our estimates are therefore drawn up for the larger output.

Equipment.

A plant with a capacity of 7,000 to 9,000 rooted cuttings a year would consist of six banks or "batteries" each of 12 glass-lidded propagating bins, as described and illustrated in the Fifth Annual Report (and in *Tropical Agriculture* 13 (1936) 201). For commercial use the division into compartments, useful for experimental work, could be omitted. Further requirements would be four batteries of similar size, but with lower walls and calico-covered lids, used for "hardening" the cuttings after potting; a concrete floor of about 1,800 square feet, covered with a roof, half glass and half galvanized iron; and about half an acre of nursery from which the cuttings would be obtained.

The equipment described is somewhat expensive. Its actual cost must vary with district, with the materials used in construction of the roof, and

with the "finish" of the work, but a fair general estimate in Trinidad seems to be about \$3,000, excluding the cost of land occupied. If this sum is written off in ten years (which is a great deal less than the life of the structure), or in other words spread over the first 75,000 cuttings, it amounts to four cents per plant.

Materials, &c.

Apart from capital outlay and labour, expense is incurred for :

1. Calico for overhead canopies and curtains, which at ten cents a yard needs renewing about once a year.
2. Cheesecloth for covering lids.
3. Sand for the bins—virtually a capital charge as there is very little wastage.
4. Chemical preparations used for stimulating rooting.
5. Potting compost.
6. Planting baskets—three cents each.
7. Repairs to propagator lids, replacement of broken glass and rotten woodwork, painting, &c.

For some of these items it is impossible to give any estimate of general applicability—the cost of sand, for example, depends almost entirely on the length of haulage. Our own costs for materials work out at $6\frac{2}{3}$ cents, including baskets, but although this covers the chief items it ignores compost, which we found impossible to assess accurately, and repairs, which did not happen to be necessary during the period of our observations. We consider that a round figure of ten cents per cutting, including the basket, is fair.

Labour.

An equipment of six batteries has been chosen as a unit for calculation, because this should be workable by one relatively highly-paid propagator with one labouring assistant. The propagator should be able to do all the setting of cuttings, watering, and "potting" into baskets, by arranging the work on a systematic basis of so many bins emptied and refilled each day. The labourer would look after the nursery and prepare potting compost, and be available for watering in the absence of the propagator. It is to be remembered that watering has to be kept up seven days a week. Rates for suitable reliable men will vary with district. Our own labour costs on the basis adopted are almost exactly six cents per rooted cutting.

Total cost.

The total cost thus comes out at tenpence (20 cents) per cutting, made up of twopence for capital charges on equipment, fivepence for materials, and threepence for labour. We give the figure with all diffidence, admitting that it is based partly on guesswork and partly on local

conditions which will not apply everywhere, even within Trinidad. We give it because at the present time many interested planters probably have no idea whether a "cutting" costs a cent or a dollar, and if our figure is only read as "somewhere between 15 cents and 25 cents" it may still add a certain degree of precision to discussions of vegetative propagation.

Comparison with other methods.

Direct comparison with the cost of budding or of raising nursery seedlings to an equivalent plantable stage is difficult, because these operations can be carried out in several different ways on estates, according to conditions. Seedling propagation in an outdoor nursery near to the final planting site involves practically no expense except for labour drawn from the estate force, and budding under similar conditions, though it involves the employment of a more skilled man, costs almost nothing for materials.

We can say, however, that budding carried out in a central nursery away from the planting site costs us, for well-grown experimental planting material, at least as much as propagation by cuttings. The seedling stocks, sown in baskets to avoid disturbance of root systems in transplanting, occupy the covered floor for nearly a year, against the average of three months for cuttings. Thus an equivalent output, though needing no propagator batteries, would need four times the roofed area, putting up capital charges from four cents to six cents. The basket usually rots before the plant is ready for the field, and needs replacement, which largely counterbalances the saving on other materials, and as there are always four times as many plants on hand to be looked after, there is no saving on labour. Our probable costs for buds (estimated on less detailed observations than in the case of cuttings) are: Capital charges on equipment—six cents; materials—eight cents; labour—six cents; total—20 cents.

It is worth noting, too, that an apparent reduction in cost may not prove economical in the end. For example, by growing and budding the rootstocks in the open, the capital charge may be cut out, with apparent saving in a favourable season; but as soon as unfavourable weather occurs, the extra attention necessary to keep the plants vigorously growing, or the lower percentage of "takes" which will result if they are not kept healthy, soon swallows up the saving. One essential requirement of planting material is that it shall be ready when wanted, and the justification of expenditure on equipment and such materials as planting baskets is that these ensure a steady output, so far as anything in horticulture can be ensured. When this aspect is duly considered, it is probable that even the cheapest form of seedling propagation is not as cheap as it appears to be.

SOME FACTORS CONTROLLING THE YIELD OF YOUNG CACAO—III.

By F. W. COPE.

IN previous Reports (1) (2) evidence was brought forward to support the view that the presence of self-incompatible members in populations of young cacao trees leads to reduction in yield from those fields. It was shown that self-compatible trees carry greater numbers of harvestable pods than self-incompatible trees.

The observations on six plots, carrying a total of 594 seedling trees, planted on River Estate in 1933, were continued during 1938-39. Counts of flowers, six to eight weeks old cherelles, wilted cherelles and mature pods were made every fortnight.

Experimental Results.

The tables presented give the combined figures for the seasons July 1937 to July 1938, July 1938 to July 1939. The conclusions which can be drawn from them will therefore bear rather more weight than those deduced from the single season's figures presented in the previous Report. The entries given in the tables are the mean values for individual sub-plots and they have been treated by Fisher's method of analysis of variance, any significant factor being pointed out in the text.

(1) FLOWERING INTENSITY PER TREE; PLOTS 1A-6C; FOR COMPARISON WITH TABLE ON p. 9 OF EIGHTH REPORT. FIGURES GIVE AVERAGE PER SEASON.

Plot.	CONTROL SUB-PLOT.		P SUB-PLOT.		K SUB-PLOT.		PK SUB-PLOT.	
	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.
1A ...	4,875	10,701	6,753	5,243	3,578	9,425	5,496	14,444
2D ...	2,338	1,690	3,063	2,522	3,316	860	4,065	5,105
3E ...	5,287	4,813	5,298	8,879	6,205	4,626	4,438	9,397
4B ...	5,840	2,067	3,545	3,020	2,845	5,639	4,417	7,149
5F ...	3,165	11,200	3,803	1,180	3,891	8,387	2,497	9,140
6C ...	2,763	1,855	3,717	5,119	5,274	4,109	3,585	3,270
Totals ...	24,268	32,326	26,179	25,943	25,109	33,046	24,498	48,505

The flowering intensities of the six plots vary widely and the differences, which show high significance, are most probably to be ascribed to the different genetic composition of the trees of each plot.

Potassium just fails to exhibit a significant effect ($p=0.05$) in enhancing flowering intensity, and it is noteworthy that the effect over two seasons is much more nearly significant than for the single season, 1937-38 (*loc. cit.* p. 10). It would be interesting to see whether several season's accumulative potash manuring would lead to a significant effect upon flowering intensity.

As during the first experimental season, the self-incompatible members of the plots produce statistically significantly more flowers than the self-compatible trees.

(2) CHERELLES SET, PER 10,000 FLOWERS BORNE; FOR COMPARISON WITH TABLE ON p. 10 OF EIGHTH REPORT.

Plot.	CONTROL SUB-PLOT.		P SUB-PLOT.		K SUB-PLOT.		PK SUB-PLOT.	
	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.
1A ...	84.1	20.7	58.0	16.7	88.7	30.1	75.6	29.7
2D ...	153.4	23.7	48.0	22.8	78.1	0.0	132.5	47.0
3E ...	68.5	16.9	85.7	39.5	89.0	34.7	61.5	35.5
4B ...	70.2	27.1	111.0	72.4	88.8	28.4	97.2	29.2
5F ...	67.9	15.2	117.0	8.5	91.7	18.5	116.2	20.8
6C ...	108.3	21.6	156.6	50.6	122.9	17.2	109.5	7.6
Totals ...	552.4	125.2	576.3	210.5	559.2	128.9	592.5	169.8

The only feature of statistical significance lies in the difference in behaviour of self-compatible and self-incompatibles. Owing to a large error variance, manurial effects are without statistical significance, although phosphorus does show a tendency towards exerting a beneficial effect upon the numbers of flowers which eventually win through to become six weeks old cherelles.

(3) CHERELLES SET, PER TREE ; FOR COMPARISON WITH TABLE ON p. II OF EIGHTH REPORT. FIGURES GIVE AVERAGE PER SEASON.

Plot.	CONTROL SUB-PLOT.		P SUB-PLOT.		K SUB-PLOT.		PK SUB-PLOT.	
	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.
1A ...	41.0	22.1	39.2	8.8	31.8	28.4	40.1	42.9
2D ...	35.9	4.0	14.7	5.8	25.9	0.0	53.9	24.0
3E ...	36.2	8.2	45.4	35.1	55.2	16.1	27.3	33.4
4B ...	41.0	5.6	39.4	21.9	25.3	16.0	43.0	20.9
5F ...	21.5	17.0	44.5	1.0	35.7	15.5	29.0	19.0
6C ...	30.0	4.0	58.2	26.0	65.0	7.1	39.3	2.5
Totals ...	205.6	60.9	241.4	98.6	238.9	83.1	232.6	142.7

A large error variance again makes it impossible to ascribe any statistical significance to the effect of potassium and phosphorus, alone or in combination, on the numbers of six weeks old cherelles appearing on the trees.

The self-compatible trees of the six plots greatly surpass their self-incompatible neighbours in the numbers of cherelles set. The difference is statistically significant to a high degree.

(4) PERCENTAGE WILT OF CHERELLES OVER SIX WEEKS OLD ; FOR COMPARISON WITH TABLE ON p. II OF EIGHTH REPORT.

Plot.	CONTROL SUB-PLOT.		P SUB-PLOT.		K SUB-PLOT.		PK SUB-PLOT.	
	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.
1A ...	70.9	47.5	62.5	53.1	33.3	52.9	70.6	61.5
2D ...	67.3	62.5	59.4	76.3	66.1	42.7	73.1	24.6
3E ...	71.5	44.3	70.0	67.8	71.1	57.4	48.6	55.0
4B ...	75.3	71.4	84.4	52.7	78.3	48.6	69.7	59.4
5F ...	71.7	78.4	84.0	66.0	69.1	53.8	75.0	65.8
6C ...	69.4	37.5	76.7	64.7	70.2	46.4	68.8	40.4
Totals ...	426.1	341.6	437.0	380.6	388.1	301.8	405.8	306.7

The self-compatible trees lose a greater proportion of cherelles through wilt than the self-incompatible trees. This difference is highly significant.

Potash promotes a statistically significant reduction in wilting intensity. Phosphorus, on the contrary, appears actually to increase the severity of wilting. The plot effect is significant, as might be expected from the rising totals for the first five plots ; it is, however, not possible to decide whether the variation is ascribable to genetic constitution or to a soil fertility gradient.

Small reductions in percentage wilt give large increases in harvested pods. The reduction, for instance, from 71.0 per cent. on the control sub-plots (self-compatibles) to 64.7 per cent. on the potash-manured sub-plots (self-compatibles), while small in itself, realises a 22 per cent. increase in harvested pods from the manured sub-plots over the control sub-plots.

(5) PODS HARVESTED, PER TREE ; FOR COMPARISON WITH TABLE ON p. 12 OF EIGHTH REPORT. FIGURES GIVE AVERAGE PER SEASON.

Plot.	CONTROL SUB-PLOT.		P SUB-PLOT.		K SUB-PLOT.		PK SUB-PLOT.	
	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.
1A ...	10.2	10.3	13.3	3.9	17.8	11.2	10.4	16.2
2D ...	8.8	0.5	3.6	1.0	6.7	0.0	9.1	13.5
3E ...	8.0	4.7	12.7	9.2	12.7	5.4	11.3	10.9
4B ...	7.5	1.1	5.2	9.9	4.1	6.0	9.8	7.7
5F ...	5.4	3.5	6.2	0.0	9.4	5.4	4.6	4.0
6C ...	6.8	2.5	9.7	7.8	20.0	3.2	9.3	1.3
Totals ...	46.7	22.6	50.7	31.8	70.7	31.2	54.5	53.6

The effects of plots and potassium manuring are highly significant. Self-compatible trees surpass self-incompatible trees with significantly higher pod yields, and on the whole, the interaction between manures and compatibility is statistically insignificant.

The beneficial effects of application of potassium salts to the soil on final pod yield would appear, therefore, to have their origin in increased intensity of flowering and decreased intensity of cherelle wilt, with emphasis on the latter factor.

(6) PODS PER 10,000 FLOWERS ; FOR COMPARISON WITH TABLE ON p. 13 OF EIGHTH REPORT.

Plot.	CONTROL SUB-PLOT.		P SUB-PLOT.		K SUB-PLOT.		PK SUB-PLOT.	
	Compa- tibles	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.	Compa- tibles.	Incompa- tibles.
1A ...	20.9	9.6	19.6	7.4	49.6	11.9	19.5	11.2
2D ...	37.4	3.0	11.8	4.0	20.1	0.0	22.3	26.4
3E ...	15.1	9.7	23.9	10.3	20.5	11.6	25.5	11.5
4B ...	12.8	5.3	14.2	32.6	14.4	10.6	22.2	10.7
5F ...	17.1	3.1	16.2	0.0	24.2	6.4	18.4	4.4
6 C ...	24.6	13.5	25.9	15.2	37.9	7.4	25.8	3.8
Totals ...	127.9	44.2	111.6	69.5	166.7	47.9	133.7	68.0

The expression of pod production in proportion to flowering eliminates the effect of flowering intensity upon a tree's cropping performance. It is interesting to note that on this basis potassium no longer exerts a significant effect and hence the contention of the preceding paragraph receives support.

Table 7 lists the values of p obtained in the analyses of variance relating to the contents of Tables 1 to 6.

Table 7.

			Plots.	K.	P.	PK.	Compatibles vs. Incompatibles.	Compatibility- Manures.
Flowering intensity	...		<0.01	> 0.05	—	—	> 0.01 <0.05	—
Cherelles set	...		—	—	—	—	<0.01	—
% cherelle set	...		—	—	—	—	<0.01	—
% wilt	...		0.05	0.01	—	—	<0.01	—
Pods harvested	...		<0.01	<0.01	—	—	<0.01	—
% pods	...		—	—	—	—	<0.01	—

Conclusions.

1. In flower production, set of cherelles, wilting of cherelles and pod production young self-compatible trees and young self-incompatible trees show statistically significant differences. A high intensity of flower production and a low intensity of cherelle wilt are features of the self-incompatible trees, yet the intensity of cherelle setting is so low that these favouring features do not lead to pod yields as good as those of the self-compatible trees.

2. The addition of potassium salts to the naturally potash deficient soils in which the young trees under observation are growing results in statistically significant increases in numbers of harvested pods, occasioned largely by a significant reduction in cherelle loss from wilt and, to a lesser extent, by a non-significant increase in flower production.

3. Self-compatible and self-incompatible trees, as manifested by the non-significant interaction between compatibility and manures, appear to react similarly towards manures.

Comparison of the Two Seasons, 1937-38 and 1938-39.

Records show that the cacao crop for the 1938-39 season, for Trinidad in general and for River Estate in particular, was a poor one.

Since there were available figures for flower production, cherelle setting, cherelle wilting and pods harvested from the trees of six plots of young seedling trees at River Estate it was possible to investigate the reactions of these young trees to the different conditions of the two seasons.

Table 8 shows the crops of mature pods for the seasons December 1937 to December 1938, and December 1938 to December 1939 to be almost identical. This result is in accord with the observation made above, since under normal circumstances a season's picking from trees just come into bearing would be larger than the previous year's.

Table 8.

PODS HARVESTED, PER TREE, DURING THE SEASONS 1937-38, 1938-39.

	CONTROL SUB-PLOT.		P SUB-PLOT.		K SUB-PLOT.		PK SUB-PLOT.		Totals.
	1937-38	1938-39	1937-38	1938-39	1937-38	1938-39	1937-38	1938-39	
Self-compatibles	8.9	7.2	8.1	9.3	11.2	12.9	9.3	9.4	76.3
Self-incompatibles	3.4	4.6	4.5	7.0	5.8	4.8	10.7	7.4	48.2
Totals ...	12.3	11.8	12.6	16.3	17.0	17.7	20.0	16.8	124.5

The analysis of these figures is presented below.

Variance due to:	Degrees of freedom.	S.S.	M.S.	$\frac{V_1}{V_2}$	$\log_e \sqrt{\frac{V_1}{V_2}}$	p.
Total ...	15	109.0				
Whole sub-units ...	7	95.6				
Compatibility ...	1	49.3	49.3	6.847	0.962	circa 0.05 barely significant.
K ...	1	21.4	21.4	2.972	0.545	
P ...	1	2.9				
PK ...	1	0.5				
Error (a) ...	3	21.5	7.2			
Year ...	1	0.0	0.0			
Year/manures ...	3	6.2	2.1			
Year/compatibility ...	1	0.3	0.3			
Error (b) ...	3	6.9	2.3			

Only the difference between compatibles and incompatibles in pod production carries any statistical significance. The zero variance for the two different years indicates identical, or almost identical, pod yields in successive seasons. Manurial effects are here without significance, due no doubt to the abridgment of the experimental data and consequent reduction in numbers of degrees of freedom.

When, however, attention is given to the stages which lead to pod production, considerable seasonal differences emerge in the trees' behaviour. Flowering intensities are given in Table 9.

Table 9.

AVERAGE FLOWER PRODUCTION, PER TREE, DURING THE SEASONS 1937-38, 1938-39.

	CONTROL SUB-PLOT.		P SUB-PLOT.		K SUB-PLOT.		PK SUB-PLOT.		Totals.
	1937-38	1938-39	1937-38	1938-39	1937-38	1938-39	1937-38	1938-39	
Self-compatibles ...	3,602	4,489	3,582	5,125	3,466	4,914	3,155	4,818	33,151
Self-incompatibles ...	4,429	6,346	3,440	5,215	4,971	6,092	6,850	7,794	45,137
Totals ...	8,031	10,835	7,022	10,340	8,437	11,006	10,005	12,612	78,288

Variance due to:	Degrees of freedom	S.S. $\times 10^{-2}$	M.S. $\times 10^{-2}$	$\frac{V_1}{V_2}$	$\frac{1}{2} \log_e \frac{V_1}{V_2}$	p.
Total ...	15	26,896.0				
Whole sub-units ...	7	18,394.6				
compatibility ...	1	8,979.0	8,979.0	4.687	0.7724	> 0.05
K ...	1	2,158.8	2,158.8			
P ...	1	174.3				
PK ...	1	1,367.8				
Error (a) ...	3	5,747.7	1,915.9			
Year ...	1	7,977.8	7,977.8	55.057	2.004	< 0.01
Year/manures ...	3	88.9				Significant
Year/compatibility ...	1	2.9				
Error (b) ...	3	434.8	144.9			

In this analysis, due probably to the reduced number of degrees of freedom involved, the difference in flowering intensities between self-compatibles and self-incompatibles is not statistically significant. There is, however, a highly significant difference between the numbers of flowers produced during the two seasons. Increase in age has been accompanied by increase in flowering intensity and, from the negligible variance for interaction between season and compatibility, both classes of tree have increased their flower production by the same extent.

Table 10.

CHERELLES SET, PER TREE, DURING THE SEASONS 1937-38, 1938-39.

	CONTROL SUB-PLOT.		P SUB-PLOT.		K SUB-PLOT.		PK SUB-PLOT.		Totals.
	1937-38	1938-39	1937-38	1938-39	1937-38	1938-39	1937-38	1938-39	
Self-compatibles ...	38.4	30.1	35.5	47.0	35.1	45.1	36.4	41.7	309.3
Self-incompatibles ...	7.6	12.6	11.8	21.0	10.7	16.9	21.1	23.3	125.0
Totals... ..	46.0	42.7	47.3	68.0	45.8	62.0	57.5	65.0	434.3

Variance due to:	Degrees of freedom.	S.S.	M.S.	$\frac{V_1}{V_2}$	$\frac{1}{2} \log_e \frac{V_1}{V_2}$	p.
Total ...	15	2576.3				
Whole sub-units ...	7	2335.1				
compatibility ...	1	2122.9	2122.9	119.3	2.3471	< 0.01 Significant.
K ...	1	43.3				
P ...	1	106.6	106.6	5.989	0.8948	> 0.05
PK ...	1	8.8				
Error (a) ...	3	53.5	17.8			
Year ...	1	105.6	105.6	17.60	1.4349	> 0.01 < 0.05
Year/manures ...	3	116.6	38.9	6.483	0.9345	Significant.
Year/compatibility ...	1	1.1				> 0.05
Error (b) ...	3	17.9	6.0			

In the second season, 1938-39, the number of cherelles set is greater than in the previous season, and, as in the case of flower production, both self-compatibles and self-incompatibles react in the same way, as indicated by the very small variance for year and compatibility interaction.

Manures are without significant effect. Phosphorus exhibits a tendency, however, towards promoting retention of cherelles from the time of fertilization up to an age of six to eight weeks. It will be recalled that it is at this age that the cherelle is recorded as a "set".

Table 11.

CHERELLES SET PER 10,000 FLOWERS BORNE, PER TREE, DURING 1937-38 AND 1938-39.

	CONTROL SUB-PLOT.		P SUB-PLOT.		K SUB-PLOT.		PK SUB-PLOT.		Total.
	1937-38	1938-39	1937-38	1938-39	1937-38	1938-39	1937-38	1938-39	
Self-compatibles ...	86.3	67.5	109.5	91.3	105.1	87.0	118.5	86.9	752.1
Self-incompatibles	24.8	20.4	34.0	35.4	18.6	24.6	26.1	29.1	213.0
Totals ...	111.1	87.9	143.5	126.7	123.7	111.6	144.6	116.0	965.1

Variance due to:			Degrees of freedom.	S.S.	M.S.	$\frac{V_1}{V_2}$	$\frac{1}{2} \log_e \frac{V_1}{V_2}$	P.
Total	...	...	15	20,244.9				
Whole sub-units	...	...	7	19,206.0				
Compatibility	...	...	1	18,164.3	18,164.3	192.2	2.629	<0.01 Significant.
K	...	...	1	44.8				
P	...	...	1	582.1	582.1	6.16	0.9091	> 0.05
PK	...	...	1	131.4				
Error (a)	...	...	3	283.4	94.5			
Year	...	...	1	407.8	407.8	21.69	1.538	> 0.01 <0.05 Significant.
Year/manures	...	...	3	38.3				
Year/compatibility	...	...	1	536.4	536.4	28.53	1.675	0.01 Significant.
Error (b)	...	...	3	56.4	18.8			

The proportion of flowers set is significantly less in the 1938-39 season than in the 1937-38 season; for although the number of flowers borne and the number of actual sets are higher during 1938-39 than in the previous season, intensity of flowering has shown greater increases than setting of cherelles. In other words, efficiency of pollination has been reduced in the second season.

There is, furthermore, a significant interaction between compatibility and season. During 1938-39 self-compatible trees set a smaller proportion of flowers than during 1937-38, while the reverse obtains for the self-incompatible trees. Pollination efficiency, it would appear, is decreased on the self-compatible trees and increased on the self-incompatible trees during the unfavourable season 1938-39. In the light of our present theory concerning the agents effecting pollination in cacao, this statement would imply that the flying insects responsible for the setting on self-incompatible trees are more efficient during 1938-39 than during 1937-38 and that the agents effecting self-pollination on the self-compatibles are less so. It would seem, therefore, that during the unfavourable second season the crawling type of pollinating insect is absent or much reduced in numbers, while the winged pollinator is just as abundant, or even more so.

Table 12.

AVERAGE PERCENTAGE WILT OF CHERELLES OVER 6 WEEKS OF AGE DURING 1937-38 AND 1938-39.

	CONTROL SUB-PLOT.		P SUB-PLOT.		K SUB-PLOT.		PK SUB-PLOT.		Total.
	1937-38	1938-39	1937-38	1938-39	1937-38	1938-39	1937-38	1938-39	
Self-compatibles ...	68.9	71.8	66.3	78.7	58.5	69.5	63.2	70.3	547.2
Self-incompatibles	63.4	50.4	56.8	64.0	41.4	57.8	43.0	54.9	431.7
Totals ...	132.3	122.2	123.1	142.7	99.9	127.3	106.2	125.2	978.9

Variance due to:	Degrees of freedom.	S.S.	M.S.	$\frac{V_1}{V_2}$	$\frac{1}{2} \log_e \frac{V_1}{V_2}$	P.
Total ...	15	1,505.4				
Whole sub-units ...	7	1,077.3				
Compatibility ...	1	763.1	763.1	80.70	2.195	<0.01 Significant.
K ...	1	279.2	279.2	30.25	1.705	0.01 Significant.
P ...	1	7.0				
PK ...	1	0.3				
Error (a) ...	3	27.7	9.2			
Year ...	1	231.0	231.0	14.80	1.347	> 0.01 <0.05 Significant.
Year/manures ...	3	148.2	49.4	3.16	0.576	
Year/compatibility ...	1	2.1				
Error (b) ...	3	46.8	15.6			

During the unfavourable 1938-39 season, cherelle wilt is significantly more severe than during 1937-38. This increase in wilting intensity is exhibited in equal degree by both self-compatible and self-incompatible elements of the population, indicated by the negligible variance for season and compatibility interaction.

Potash application to the soil significantly reduces intensity of wilting; and the effect is the same in both seasons, there being no significant interaction between season and manures.

Table 13 summarises the result of the preceding tables.

Table 13.

VALUES OF 'P' EXTRACTED FROM TABLES 8-12.

	Compati- bility.	K.	P.	PK.	Year.	Year/ Manures.	Year/Com- patibility.
Flowering intensity	—	—	—	—	<0.01	—	—
Cherelles set ...	<0.01	—	—	—	> 0.01 <0.05	—	—
Per cent. Cherelle set	<0.01	—	—	—	> 0.01 <0.05	—	0.01
Wilt ...	<0.01	0.01	—	—	> 0.01 <0.05	—	—
Harvested pods ...	0.05	—	—	—	—	—	—

It would appear from the results quoted in this paper that during the unfavourable season, 1938-39 (as judged by Trinidad crop returns), the young cacao trees under observation received their set-back as a result of increased wilting; for had the season been an average one, the observed increase in flowering and setting intensities would presumably have contributed towards a crop greater than in the previous year.

Summary.

1. The pod production of young seedling cacao trees is shown to be identical in two consecutive seasons, the first of which was favourable to cropping and the second, unfavourable.

2. In the unfavourable year, flowering intensity is increased in both self-compatible and self-incompatible trees. The increase is statistically significant.

3. In the unfavourable year, cherelles set in greater numbers on both classes of tree than in the previous year. The seasonal difference is statistically significant.

4. In the unfavourable year, less cherelles are set from 10,000 flowers in the self-compatible trees, but more per 10,000 flowers in the self-incompatibles, than in the previous season. The interaction between compatibility and season is statistically significant.

5. In the unfavourable year, wilting of cherelles is significantly more severe than in the favourable season.

References.

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- (2) Voelcker, O. J. and Cope, F. W.—Some Factors controlling the Yield of Young Cacao—I. *Seventh Ann. Rep. on Cacao Res.* (1937), 1938.

AGENTS OF POLLINATION IN CACAO.

By F. W. COPE.

A HIGH degree of cross-pollination has been proved to exist amongst the cacao population of Trinidad (9). The regular occurrence of high-yielding self-incompatible trees in the field and the incidence of purple beans in pods from pure Criollo cacao trees in Forastero fields are further indisputable evidence that cross-pollination takes place in cacao.

The work of Harland (4), Stahel (8) and Posnette (6) has shown both flying and crawling insects to be involved in the process of pollen transference. In Java, wind pollination appears to be the accepted theory (3); but Harland (4) gives adequate reasons for regarding wind pollination as most unlikely.

The insects commonly associated with cacao flowers are thrips*, red ants†, and aphides‡. Uzel, as mentioned by Wright (11), from experiments made in Ceylon, favoured thrips as the pollinating agents. Jones (5) in Dominica, concluded that ants, attracted by aphides, were responsible. Harland (4) reached the conclusion that in the material he studied ants and aphides were together important agents of pollination. Pound (7) mentions that "general setting in 1932 was associated with the time when the flowers became thrips-infested".

It was considered that the young seedling trees at River Estate (10, 1), upon which frequent observations of flowering and cherelle setting intensities were being made, would provide good material for an analysis of the connection, if any, between setting and insect visits. Additional observations were therefore undertaken which involved counts of thrips, red ants and aphides found in flowers picked from selected trees.

Immediately after the fortnightly counts of flowers, new cherelles, wilted cherelles and pods had been made for the 99 trees of Plot 1A, ten flowers were picked from each of 17 members of the plot, the picking being performed at the same time in the day (10 a.m.) on every occasion and at random over the accessible portions of these still small trees. Eight self-compatible and nine self-incompatible trees were regularly visited.

The flowers were gently picked from the trees with forceps and immediately plunged into 70 per cent. alcohol contained in a labelled tube held just below the flower. In this way loss of insects resulting from manipulation of the flowers was reduced to a minimum. The tubes were then well corked, taken to the laboratory and the insects carefully dissected out from the flowers. Thrips, red ants, aphides and winged aphides were separately counted and recorded.

Experimental Results.

Tables 1, 2 and 3 present the numbers of thrips, red ants and aphides collected over a period of 14 months. A table for winged aphides is not given since these insects appeared only very infrequently and in very small numbers amongst the collections.

From the Tables it is clear that aphides are by far the most abundant insects present: thrips are of less frequent occurrence and red ants are often completely lacking in the fortnightly collections.

In the case of thrips and red ants, the self-incompatible trees which were examined carry a higher proportion of both of these insects than do the self-compatible trees, although on a statistical basis the differences lack any great significance. The aphid densities for both groups of trees are almost identical. It would seem that the self-incompatible trees are favoured by the most mobile of the insects under consideration, the winged thrips, less so by the red ant and not at all by the sedentary aphid. If there actually exists such a positive attraction for the thrips to the self-incompatible tree it can only readily be ascribed to the high flowering intensity which is characteristic of the majority of such trees.

Table 4 and Figure 1 show the fortnightly densities of setting and of insects in self-compatible and self-incompatible members of Plot 1A. The numbers quoted for the three classes of insects are given against the dates of collection: the entries for setting intensities are calculated from the numbers of flowers occurring on the trees on the day of collection and the numbers of cherelles counted eight weeks later, these two numbers being obtained in the fortnightly routine counts.

The two setting curves are derived from figures for all the self-compatible and self-incompatible members, respectively, of Plot 1A, 99 trees in all. The curves of insect densities are obtained from 17 members.

The curves for setting show two maxima, in early July, 1938, and mid-December, 1938. From January 1939 to May 1939, there is setting of a very low intensity. The curves of thrips and red ant densities show similar maxima at the same times in the season, which is suggestive of a connection between the presence of these two insects and pollination. Curves of aphid density and setting intensity are not in any way similar: indeed, aphides seem to attain their maximum numbers when setting is at its minimum intensity.

* *Frankliniella parvula* Hood.

† *Wasmannia auropunctata* Rog.

‡ *Toxoptera aurantii* B. de Fonsc.

Table 1.

THRIPS DENSITIES.

Date.	SELF-COMPATIBLE TREES.								SELF-INCOMPATIBLE TREES.								Thrips per 100 flowers.	
	1A 1	1A 32	1A 34	1A 50	1A 57	1A 63	1A 87	1A 93	1A 5	1A 12	1A 16	1A 24	1A 44	1A 60	1A 62	1A 76		1A 95
9. 4.38 ...	0	0	—	—	4	0	—	0	0	1	0	0	0	0	0	0	1	4.3
23. 4.38 ...	0	0	—	0	0	0	—	3	1	0	1	0	2	1	2	2	0	8.0
7. 5.38 ...	0	0	—	—	4	—	0	2	0	0	1	0	5	3	0	1	2	12.9
21. 5.38 ...	0	2	0	0	1	0	0	0	0	0	0	0	0	1	2	1	0	4.1
6. 6.38 ...	0	—	—	1	0	0	1	0	4	0	0	0	1	0	0	1	3	7.3
20. 6.38 ...	2	0	0	1	0	0	0	1	3	1	0	0	3	0	1	1	2	8.8
4. 7.38 ...	—	1	0	1	2	—	0	—	3	0	1	0	0	0	4	2	3	12.1
18. 7.38 ...	0	0	4	0	—	—	—	0	0	0	1	0	—	—	0	0	1	5.0
22. 8.38 ...	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	2	3.5
10. 9.38 ...	0	1	1	1	1	0	—	0	0	0	3	1	0	—	2	1	3	9.3
19. 9.38 ...	—	1	0	0	0	—	0	0	1	0	1	0	0	0	1	0	1	3.3
4.10.38 ...	—	0	0	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0.0
18.10.38 ...	—	2	2	2	0	2	2	4	0	3	8	2	0	—	5	6	0	25.3
1.11.38 ...	0	0	0	0	0	0	0	4	2	0	9	2	1	—	1	1	0	12.5
15.11.38 ...	0	6	0	3	1	0	0	3	1	2	1	1	1	2	7	6	3	21.8
30.11.38 ...	0	0	1	3	3	—	0	1	—	1	2	0	0	2	11	2	2	18.7
14.12.38 ...	—	1	2	0	4	0	—	4	1	4	5	1	0	—	6	3	3	24.3
3. 1.39 ...	0	1	0	0	1	0	—	—	0	0	0	0	2	0	0	0	0	2.7
11. 1.39 ...	0	0	0	0	0	—	—	0	0	0	0	0	0	1	0	0	0	0.7
25. 1.39 ...	0	0	0	0	0	—	—	0	0	0	0	0	0	0	0	0	0	0.0
7. 2.39 ...	0	0	—	0	0	0	—	0	0	—	0	0	0	—	0	0	0	0.0
21. 2.39 ...	0	—	—	—	1	1	—	—	1	2	0	0	0	—	—	1	0	6.0
8. 3.39 ...	0	0	—	—	0	—	—	0	0	0	0	0	—	0	0	0	2	1.7
22. 3.39 ...	0	—	—	—	2	—	—	0	0	1	2	1	—	0	4	2	0	10.9
5. 4.39 ...	2	1	0	2	2	—	—	0	2	0	4	0	0	0	1	8	3	16.7
19. 4.39 ...	—	0	—	0	1	0	—	2	1	3	0	1	0	—	1	—	2	9.2
18. 5.39 ...	0	0	—	0	—	—	0	1	0	1	1	0	1	4	0	0	1	6.4
1. 6.39 ...	3	5	—	4	—	—	0	0	1	0	3	0	0	3	3	3	1	18.6
15. 6.39 ...	0	0	0	0	1	0	0	1	1	0	4	0	0	0	1	1	1	5.9
Average per collection per 100 flowers ...	3.0	8.1	5.6	7.5	10.8	1.9	2.0	10.4	8.3	6.8	16.6	3.1	6.5	8.1	18.6	15.0	12.4	

Table 2.

RED ANT DENSITIES.

Date.	SELF-COMPATIBLE TREES.								SELF-INCOMPATIBLE TREES.									Ant Density per 100 flowers.
	1A 1	1A 32	1A 34	1A 50	1A 57	1A 63	1A 87	1A 93	1A 5	1A 12	1A 16	1A 24	1A 44	1A 60	1A 62	1A 76	1A 95	
9. 4.38 ...	0	1	—	—	0	0	—	0	1	0	0	2	0	0	0	0	1	3.6
23. 4.38 ...	0	1	—	0	0	0	—	0	2	7	0	0	0	0	0	0	2	8.0
7. 5.38 ...	0	0	—	—	0	—	0	0	0	0	0	0	0	2	0	0	0	1.4
21. 5.38 ...	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0.6
6. 6.38 ...	0	—	—	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0.7
20. 6.38 ...	0	0	0	0	0	0	1	0	0	4	1	2	1	0	1	0	1	6.5
4. 7.38 ...	—	0	0	0	0	—	0	—	0	0	2	3	1	0	0	0	0	4.3
18. 7.38 ...	0	1	1	0	—	—	—	0	1	0	2	9	—	—	0	0	0	10.8
22. 8.38 ...	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	1	0	3.5
10. 9.38 ...	0	0	0	0	0	0	0	0	0	0	0	0	0	—	0	0	1	0.6
19. 9.38 ...	—	0	0	0	0	—	—	0	0	1	0	0	0	0	0	0	0	0.7
4.10.38 ...	—	0	0	0	0	—	0	0	0	0	0	1	0	0	0	0	0	0.7
18.10.38 ...	—	0	0	0	0	0	0	0	0	0	0	1	0	—	0	0	0	0.7
1.11.38 ...	0	0	0	0	0	0	1	0	0	0	0	0	0	—	0	0	0	0.6
15.11.38 ...	0	0	0	0	1	3	1	0	1	1	0	1	1	1	0	1	0	6.5
30.11.38 ...	0	0	0	0	0	—	0	0	—	0	0	—	0	1	0	0	0	0.7
14.12.38 ...	—	0	0	0	1	4	—	0	0	0	0	7	0	—	0	0	5	12.1
3. 1.39 ...	0	0	0	0	0	0	—	—	0	0	0	8	0	0	0	1	2	7.3
11. 1.39 ...	0	0	0	0	0	—	—	0	0	0	0	0	0	0	0	0	0	0.0
25. 1.39 ...	0	0	0	0	0	—	—	0	0	0	0	0	0	0	0	0	0	0.0
7. 2.39 ...	1	0	—	0	0	0	—	0	0	—	0	—	0	—	0	0	0	0.8
21. 2.39 ...	0	—	—	—	0	0	—	—	0	0	0	0	0	—	—	0	0	0.0
8. 3.39 ...	0	0	—	—	2	—	—	0	0	0	0	0	—	0	0	0	0	1.7
22. 3.39 ...	0	—	—	—	0	—	—	0	0	0	0	0	—	0	0	0	0	0.0
5. 4.39 ...	0	0	0	0	0	—	—	0	0	0	0	0	0	0	0	0	0	0.0
19. 4.39 ...	—	0	0	0	0	—	—	0	0	0	0	0	0	—	0	—	0	0.0
18. 5.39 ...	0	0	—	0	0	—	0	0	0	0	1	0	0	0	0	0	0	0.7
1. 6.39 ...	0	0	—	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0.0
15. 6.39 ...	0	0	0	0	0	0	0	0	0	0	0	—	0	2	0	0	0	1.3
Average per collection per 100 flowers ...	0.4	1.2	0.5	0.0	1.5	4.7	2.0	0.0	1.8	5.0	2.4	13.5	2.3	2.9	0.4	1.1	4.1	

Table 3.

APHID DENSITIES.

Date	SELF-COMPATIBLE TREES.								SELF-INCOMPATIBLE TREES.									Aphid Density per 100 flowers.
	1A 1	1A 32	1A 34	1A 50	1A 57	1A 63	1A 87	1A 93	1A 5	1A 12	1A 16	1A 24	1A 44	1A 60	1A 62	1A 76	1A 95	
9. 4.38 ...	0	98	—	—	0	2	—	218	58	132	35	70	92	52	9	4	42	580.0
23. 4.38 ...	0	14	—	65	0	0	—	2	11	13	3	0	0	3	0	0	2	75.3
7. 5.38 ...	0	0	—	—	0	—	1	0	0	0	0	0	10	0	0	0	0	7.9
21. 5.38 ...	0	2	8	0	0	0	0	0	0	0	0	0	0	0	0	4	6	11.8
6. 6.38 ...	0	—	—	0	0	0	6	1	0	6	0	0	0	0	0	6	0	12.7
20. 6.38 ...	10	6	2	0	1	0	1	38	3	1	0	0	0	0	0	1	0	37.1
4. 7.38 ...	—	0	0	0	0	—	1	—	37	8	10	0	0	0	0	1	0	40.7
18. 7.38 ...	0	2	6	0	—	—	—	0	2	0	0	0	—	—	0	6	0	13.3
22. 8.38 ...	4	0	3	24	3	0	0	25	10	1	0	0	0	4	6	5	18	60.6
10. 9.38 ...	0	15	0	0	4	3	0	0	0	1	1	0	0	—	0	0	0	15.0
19. 9.38 ...	—	3	0	0	1	—	—	0	1	0	0	0	0	0	0	0	0	3.6
4.10.38 ...	—	6	1	0	0	—	0	0	0	4	2	0	0	0	1	1	4	12.7
18.10.38 ...	—	0	0	0	4	12	0	0	0	5	2	0	0	—	2	0	1	17.3
1.11.38 ...	0	0	3	1	11	0	7	8	1	1	15	0	0	—	2	13	5	41.9
15.11.38 ...	3	0	0	1	6	7	1	2	0	3	0	0	1	0	0	1	0	14.7
30.11.38 ...	25	0	3	2	0	—	0	18	—	3	0	—	1	2	0	1	0	39.3
14.12.38 ...	—	2	0	0	0	0	—	2	0	0	14	0	0	—	0	3	0	15.0
3. 1.39 ...	0	0	8	0	0	0	—	—	0	0	0	0	0	0	0	0	0	5.3
11. 1.39 ...	6	4	1	38	0	—	—	0	0	17	0	0	0	0	0	1	0	44.7
25. 1.39 ...	0	0	0	25	0	—	—	0	5	13	5	0	0	0	40	2	86	117.3
7. 2.39 ...	6	3	—	10	1	1	—	0	1	0	2	—	0	—	3	5	0	24.6
21. 2.39 ...	10	—	—	—	3	3	—	—	0	13	3	4	0	—	—	4	16	56.0
8. 3.39 ...	0	23	—	—	5	—	—	5	0	41	0	0	—	0	2	23	0	82.5
22. 3.39 ...	4	—	—	—	1	—	—	6	3	0	4	0	—	0	1	0	0	17.3
5. 4.39 ...	0	0	2	0	1	—	—	6	0	8	2	0	0	8	22	0	1	33.3
18. 4.39 ...	—	1	0	1	0	—	—	0	0	0	9	5	1	—	0	—	2	15.8
18. 5.39 ...	0	0	—	3	—	—	0	0	0	0	3	1	0	0	12	0	0	13.6
1. 6.39 ...	0	0	—	0	—	—	0	0	0	0	0	0	0	0	0	0	0	0.0
15. 6.39 ...	0	0	0	0	0	0	0	7	0	0	0	—	1	6	0	0	0	8.7
Average per collection per 100 flowers ...	29.6	68.8	19.5	70.8	15.8	18.7	11.3	135.2	47.2	96.4	37.9	30.8	40.8	35.7	35.7	28.9	63.1	

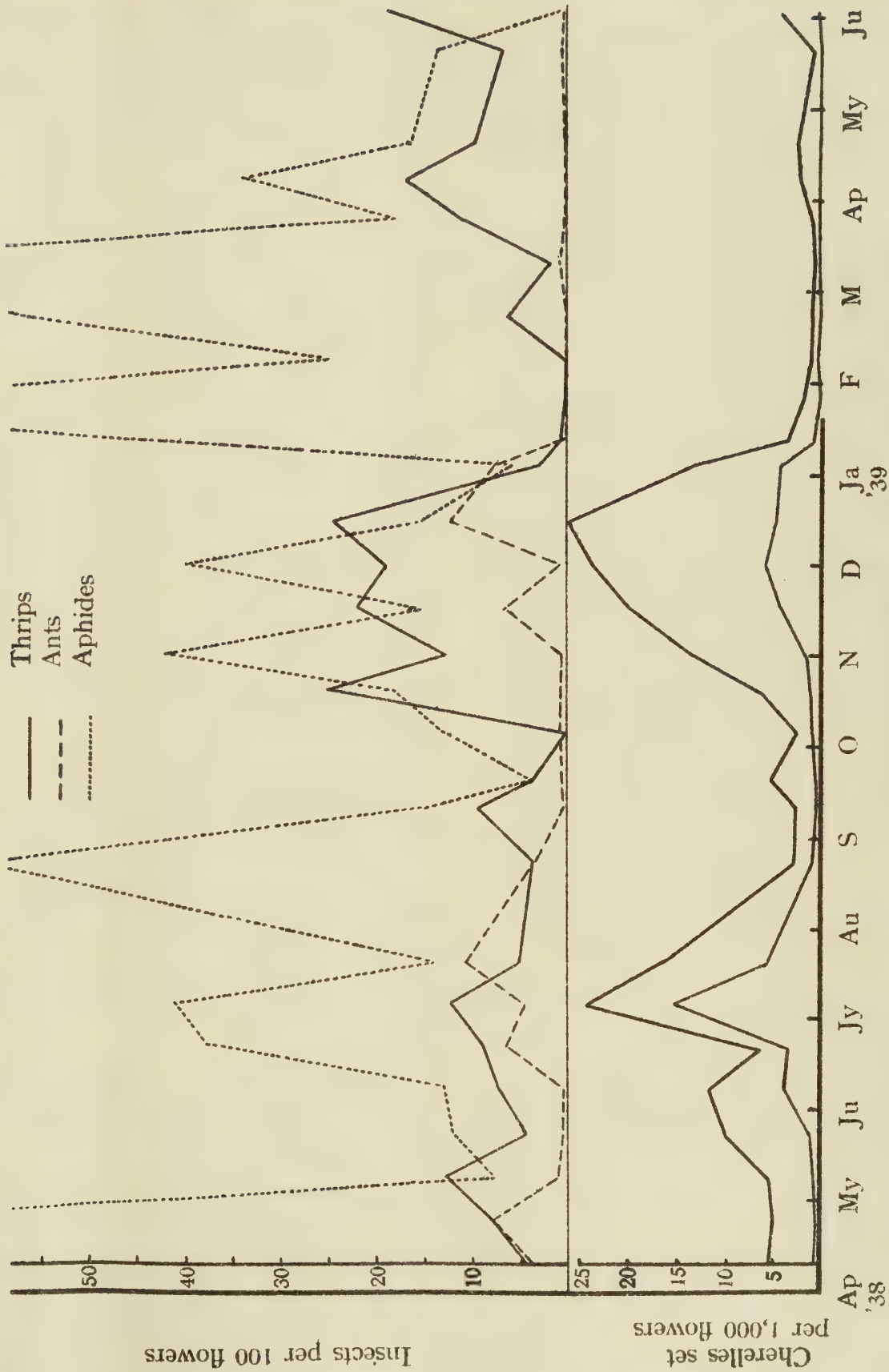


FIG. 1.—Graphs of setting intensities and insect densities. Self-incompatibles setting curve below, self-compatibles above.

Table 4.
SETTING AND INSECT DENSITIES.

Date.	Che- relles set per 10,000 flowers. Compati- bles.	Che- relles set per 10,000 flow- ers. Incom- pati- bles.	Thrips per 100 flow- ers.	Red ants per 100 flow- ers.	Aphides per 100 flowers.
9. 4.38	5.24	0.74	4.3	3.6	58.0
23. 4.38	4.95	0.59	8.0	8.0	75.3
7. 5.38	5.63	1.07	12.9	1.4	7.9
21. 5.38	10.02	1.84	4.1	0.6	11.8
6. 6.38	11.90	3.73	7.3	0.7	12.7
20. 6.38	6.08	3.12	8.8	6.5	37.1
4. 7.38	24.64	15.43	12.1	4.3	40.7
18. 7.38	16.13	5.60	5.0	10.8	13.3
22. 8.38	2.74	1.05	3.5	3.5	60.6
10. 9.38	2.59	0.59	9.3	0.6	15.0
19. 9.38	5.32	0.68	3.3	0.7	3.6
4. 10.38	2.35	1.25	0.0	0.7	12.7
18. 10.38	6.06	1.53	25.3	0.7	17.3
1. 11.38	13.15	1.97	12.5	0.6	41.9
15. 11.38	19.98	4.10	21.8	6.5	14.7
30. 11.38	23.76	5.92	18.7	0.7	39.3
14. 12.38	26.26	4.47	24.3	12.1	15.0
3. 1.39	13.10	4.15	2.7	7.3	5.3
11. 1.39	3.45	0.94	0.7	0.0	44.7
25. 1.39	1.67	0.0	0.0	0.0	117.3
7. 2.39	1.19	0.46	0.0	0.8	24.6
21. 2.39	1.25	0.0	6.0	0.0	56.0
8. 3.39	0.86	0.22	1.7	1.7	82.5
22. 3.39	1.35	0.12	10.9	0.0	17.3
5. 4.39	2.02	0.07	16.7	0.0	33.3
19. 4.39	2.46	0.11	9.2	0.0	15.8
18. 5.39	1.45	0.09	6.4	0.7	13.6
1. 6.39	4.05	0.72	18.6	0.0	0.0
Totals ...	219.65	60.56	254.1	72.5	1409.3

Table 5.

	Correlation between	r	$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$	p
Self-compatibles	Setting intensity and red ant density	+0.5794	3.624	<0.01
	Setting intensity and thrips density	+0.5089	3.014	<0.01
	Setting intensity and aphides density	-0.1160	0.636	0.55
Self-incompatibles	Setting intensity and red ant density	+0.4259	2.400	0.02
	Setting intensity and thrips density	+0.2569	1.355	0.20
	Setting intensity and aphides density	-0.1115	0.572	0.60

Calculations of correlation coefficients add weight to the above findings. Table 5 shows that there is good positive correlation between setting intensity on both self-compatible and self-incompatible trees and densities of red ants and thrips; and there is a low negative correlation between setting and aphid density.

Taking all the 99 trees together and both red ants and thrips together, the value of 'r' so obtained is $\times 0.5906$. It is therefore suggestive that setting of cherelles in the cacao trees of this plot is closely connected with the combined densities of red ants and thrips. When these two insects are abundant, setting is intensified; on their disappearance (as in January, 1939) setting intensity at once falls to a low level.

A study of the curves for setting and densities of thrips and red ants (Fig. 1) reveals some interesting facts. In the case of thrips, their density over the months of May, June and July (of 1938) is more or less uniform, with fluctuations which could be expected from a study of such a population of insects, yet setting rises progressively and reaches a peak value in early July. Conversely, in February, March and April (of 1939) setting of cherelles is uniformly of a very low intensity, yet the density of thrips rises rapidly and in April the flowers are receiving numerous thrips visitors. It has, however, been explained earlier in this paper that the figures for setting are derived from counts of cherelles about eight weeks old (it is impracticable to count cherelles younger than this) and therefore the important losses amongst cherelles due to wilt during the first eight weeks of their lives have to be neglected. In other words a high intensity of actual setting may be masked by a high rate of cherrille loss. It has been shown, furthermore (2) that there is a seasonal variation in success to be expected from hand self-pollinations and presumably therefore, also from insect pollinations. From the figures quoted in Table V (*ibid.*, p. 16) for percentage setting for the months of May, June and July (of 1938) it can be seen that the rising degrees of success from hand self-pollinations might well explain the rising success of insect pollination, even allowing the numbers of thrips to remain more or less constant. Many other factors apart from the merely mechanical process of pollen deposition upon the stigma will be called into play in determining what proportion of flowers on a cacao tree will eventually mature into cherelles eight weeks old, to which the figures quoted for setting in this paper relate.

It is easy to ascribe self-pollinations to red ants, for these small insects can be observed moving actively about the cacao flower. In some cases the insect in the flower has been seen to be dusted with yellow pollen. But only on self-compatible trees would its pollinating activities be manifested since it is difficult to believe that these insects make extensive tree to tree migrations. Hence the higher correlation between ant density and setting of self-incompatible trees than between

ant density and setting of self-compatible trees, as shown in Table 5, is an anomaly. It is, however, apparent from these studies that neither the thrips nor the red ant can alone be identified as the agent of pollination in cacao. A more convincing argument in favour of insect pollination is derived from a study of setting which includes both insects, more or less as equally effective pollinators.

A problem of direct interest arises from the figures quoted for thrips and red ants densities for different times throughout the year. The sudden increase in the numbers of thrips to be found in cacao flowers in October, 1938, and their almost complete disappearance in mid-January, 1939, are remarkable features for which no ready explanation can as yet be given. Since this outburst of thrips is also closely connected with heavy setting it is obviously important, assuming that the connection is real, to know the factor or factors responsible for this great increase in the numbers of thrips. Red ants show similar abrupt alterations in density. Studies in the ecology of these two insects should therefore yield valuable information which might then be directed into channels designed to increase the numbers of insect visitors to the flowers of cacao.

It cannot be over-emphasised that the evidence presented in this paper is entirely circumstantial and final proof or disproof that thrips and red ants are responsible for natural pollination in cacao will involve a great deal more research.

Summary.

1. Records of observations on 99 young cacao trees with regard to the intensity of setting of cherelles have been correlated with observations of the numbers of thrips, red ants and aphides found amongst the flowers on 17 of them.

2. Good positive correlations can be established between the densities of both red ants and thrips and the intensity of cherelle setting.

3. Very low negative correlation is shown to hold between the density of aphides and the intensity of cherelle setting.

4. The evidence afforded by these results suggests, circumstantially, that red ants and thrips are the responsible agents of pollination in the cacao population studied.

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STUDIES IN THE MECHANISM OF SELF-INCOMPATIBILITY IN CACAO—II.

By F. W. COPE.

IN a short paper, presented in a previous Report (2), it was shown that failure of self-pollinations on a self-incompatible tree was not due to reduction in growth rate of the pollen-tubes in the style. It was established that no statistically significant differences existed between the growth rates of pollen-tubes in styles, pollinated compatibly and incompatibly.

It was concluded (*loc. cit.*) that since no inhibition of tube growth occurred in the style, failure of self-incompatible selfings to set fruit was due to some factor or factors operating later in the fertilization processes. It was the object of these present studies to determine at what stage the breakdown in the mechanism of fertilization took place.

Material and Methods.

The same material was used as in the previous studies. The early studies reported in this present paper were made on hand-sectioned material and at a latter stage microtome sections were used to confirm and extend the preliminary findings.

Flowers of the self-incompatible tree M1 were selfed, or crosses made with pollen from the self-compatible I.C.S. 101, as required. No emasculation or covering of the flowers was attempted since far less than one per cent. of the flowers borne on this tree ever set. A collection of 100 opened flowers at random on one occasion showed that only one stigma had received pollen, and then only to the extent of two grains. The studies reported here were extensive and the

results obtained so consistent that the omission of operations providing rigorous exclusion of possible contamination appeared fully justified.

All pollinations were made at 8 a.m., experience showing that at this time in the morning pollen was abundant and fresh. Only newly opened flowers were pollinated.

Material for hand sectioning was collected 20 to 31 hours after pollination. After dissecting out the ovary with a needle-knife, longitudinal sections of it were cut in pith and the sections subjected to gentle pressure in water under a cover slip. After squashing, the sections were bleached overnight in Eau de Javelle, prepared according to Debenham (3). Following a thorough washing in several changes of water, the sections were stained by Nebel's method (5), using lacmoid and martius yellow as the staining agents. Mounts were made in 50 per cent. glycerine containing a little lacmoid in solution.

Ovaries for microtome sectioning were collected at times ranging from $7\frac{1}{2}$ hours to 96 hours after pollination. Before cutting out the ovary from the flower, segments were removed from either side of the ovary, by tangential cuts, in order to permit rapid entry of the fixative. Amongst several tried, 'Craf' fixative (8) gave the best results. The fixed material was taken up to wax according to the schedule recommended in the paper cited, and cut at ten microns. Gentian violet was used as the staining agent, with differentiation in clove oil sufficient to leave a little stain in the cell walls.

Results.

Stained hand-sections revealed no differences in behaviour of pollen-tubes in the ovarian and placental tissues of the ovaries of M1, pollinated compatibly and incompatibly. Pollen-tubes from self-pollinations of M1 were seen to reach to the base of the stylar canal, situated at the middle of the ovary, and from thence to penetrate in all directions towards the ovules. Pollen-tubes were frequently to be seen running in the spaces between the ovules and, in a few fortunate cases, were seen actually to penetrate the ovule.

Pollen-tubes were rendered strikingly visible by the technique employed. Apart from the blue colour imparted to the tube wall by the lacmoid, the callus plugs, occurring at intervals throughout the length of the tube, made the courses of the tubes easy to follow. The spacing of these plugs became noticeably wider the deeper the tubes penetrated into the ovary.

Figure 2 is a camera-lucida drawing of a typical section showing the deep penetration of M1 pollen-tubes in the ovarian and placental tissues of its own ovaries. Figure 3 shows the penetration of a tube, apparently swollen as a result of treatment, to the tip of the nucellus.

Pollen of I.C.S. 101 yielded precisely similar figures.

There can be no doubt, therefore, that pollen-tubes, in incompatible pollinations, experience no difficulty in reaching the ovules. No inhibitory effects on the growth of pollen-tubes appear to be exerted in the style, in the ovary or in the placentae. Incompatibility in cacao seems, therefore, to differ markedly from the types of incompatibilities reported for the majority of flowering plants which exhibit it.

Microtome sections corroborated the information derived from hand-sections. Ovules, in ovaries pollinated both compatibly and incompatibly, showed abundant evidence of pollen-tube penetration. No stages earlier than $7\frac{1}{2}$ hours from pollination were studied but since at that time both compatible and incompatible tubes were seen in the nucellae of ovules, it was concluded that both types of tube had grown at approximately equal rates through the tissues of the ovary. Pollen-tube entry appeared confined to the micropylar end of the ovule.

Figure 4 shows the entry of an I.C.S. 101 pollen-tube into an M1 embryo-sac. The nuclear organization was found to be as described by Cheesman (1). The synergidae are large, vacuolate structures, at this early stage occupying fully half the embryo-sac. Each synergida carries a large, conspicuous nucleus, the nucleolus staining intensely with gentian violet; the nucleus often lies against the vacuole. The egg cell stands in close association with the synergidae and although it is a large structure, its poor contents make it less conspicuous than the synergidae. The egg nucleus, containing at this $7\frac{1}{2}$ hour stage a single conspicuous nucleolus, is usually a little smaller than the synergidae nuclei. Two other prominent objects in the embryo-sac are the polar nuclei large and rather ill-defined in extent, and carrying each a darkly staining nucleolus. Numerous starch grains, of characteristic form and appearance, complete the contents of the embryo-sac, except, occasionally, for one or more disintegrated antipodals.

In the section figured (Fig. 4), the pollen-tube has pierced the embryo-sac wall and the tip, constricted in its passage through the wall, has swollen into a pronounced vesicle.

Figure 5 shows the entry of an M1 pollen-tube into an M1 embryo-sac. The nuclear organization is exactly the same as before.

In ovules showing entry of pollen-tubes (as in Figs. 4 and 5), the synergidae appear to be invested with fringed, darkly staining caps over the micropylar ends of the two cells. No discharge of male gametes was ever seen in the sections studied.

It is obvious therefore that the embryo-sac structure in this self-incompatible cacao tree is in no way abnormal; indeed, its normality can be inferred from its satisfactory functioning with pollen from any self-compatible tree.

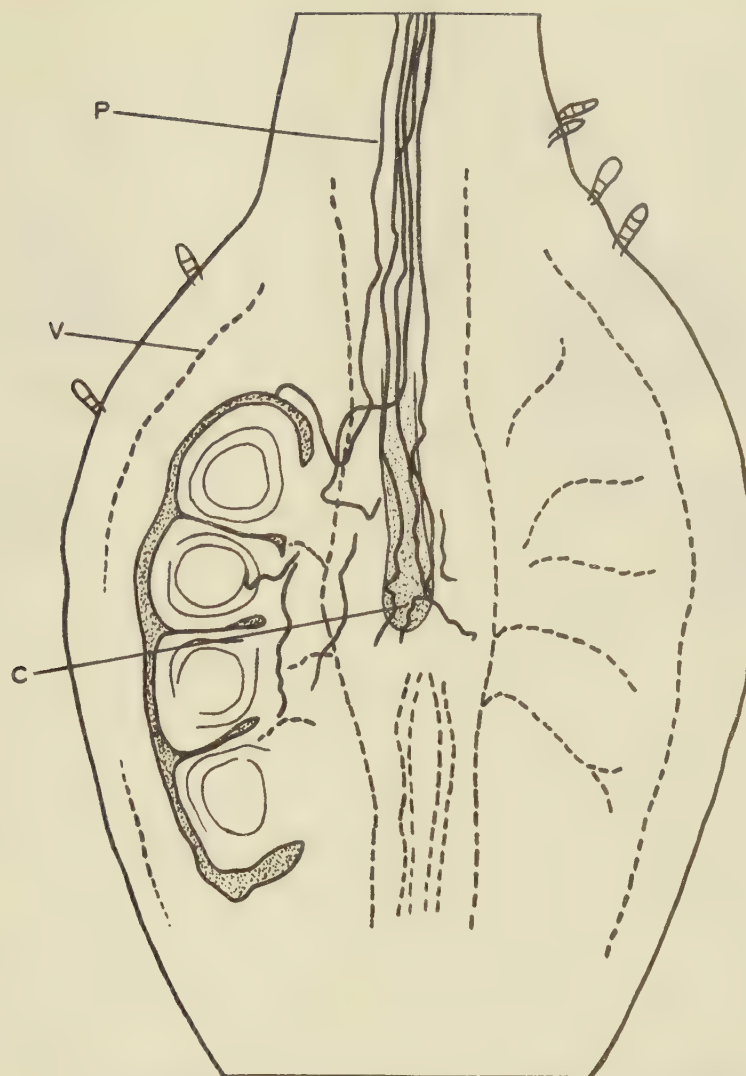


FIG. 2. Longitudinal section of selfed M1 ovary, 31 hours after pollination. P, pollen-tube; C, stylar canal; V, vascular bundle. $\times 60$

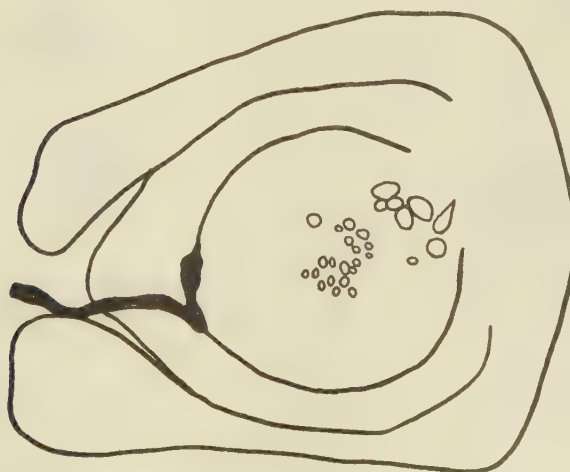


FIG. 3. Ovule showing entry of pollen-tube. M1 selfed. $\times 250$

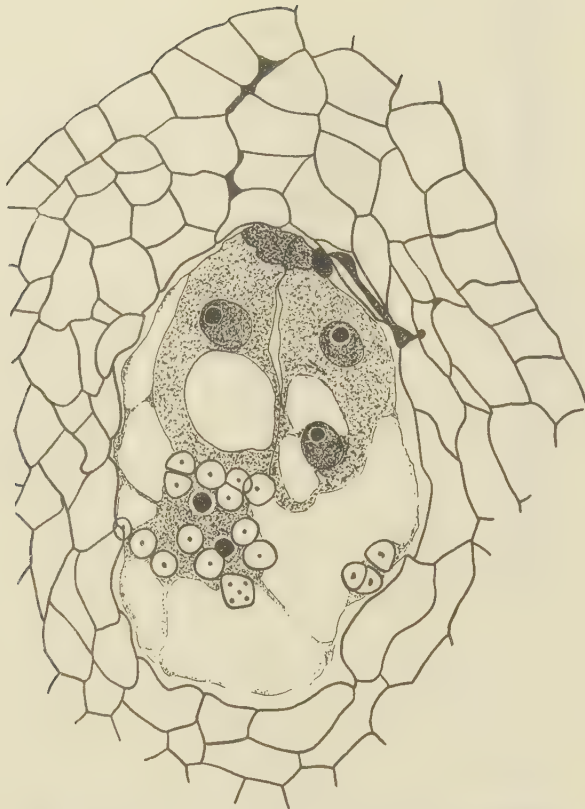


Fig. 4. M1 X I.C.S. 101. 7½ hours after pollination.
Entry of pollen-tube into embryo-sac. X 1200.



Fig. 5. M1 selfed. 7½ hours after pollination.
Entry of pollen-tube into embryo-sac.
X 1200.



Fig. 6. M1 selfed. 30 hours after pollination.
Male and female gametes side by side.
X 1200.



Fig. 7. M1 selfed. 30 hours after pollination.
Gametes fused; zygote with two nucleoli.
X 1200.

Thirty hours after pollination the egg nuclei, in ovaries receiving either M1 or I.C.S. 101 pollen, frequently showed that fertilization had been effected. In many cases the small male gamete could be seen lying in contact with the female gamete; more frequently the egg was seen to contain two deeply staining nucleoli.

Where such evidence of normal fertilization was obtained, the polar-body was always triple in structure. Three heavily staining nucleoli were now visible where before there had been two; the added nucleolus was often smaller than the others. Here, as in other stages, the polar nuclear membranes were very ill-defined and it was found impossible to decide whether or not the nuclei had fused. None the less, the nucleoli gave clear evidence of double or triple structure in the fusion nucleus.

In the majority of embryo-sacs one of the synergidae had disintegrated and all that remained of it was a darkly staining mass lying against the egg cell. In ovaries giving clear evidence of fertilization it was always possible, however, to find a few ovules which obviously had not received male gametes and here the synergidae were intact, the egg was a single structure and the polar nuclei were exactly as in the $7\frac{1}{2}$ hour stage.

In 11 M1 ovaries, at 30 hours after incompatible pollination, 64 percent. of the embryo-sacs examined gave indications that fertilization had been effected. The presence of two gametes in contact or of two nucleoli in the ovum or of three nucleoli in the polar mass was taken as sufficient evidence of fertilization.

In six M1 ovaries, at 30 hours after pollination with compatible I.C.S. 101 pollen, 80 per cent. of the embryo-sacs furnished evidence of fertilization, taking any or all of the three above mentioned criteria into consideration.

Figure 6 depicts an M1 embryo-sac from a selfed ovary. One of the synergidae has degenerated to give a deeply staining mass against the egg cell. The other synergida is intact and nucleolate. The small male gamete lies against the female gamete and the polar mass is distinctly triple in structure, the added, second male gamete possessing a nucleolus different from the two maternal nucleoli. Starch grains are numerous and large in size.

Figure 7 closely resembles the preceding one, except that here the gametes have obviously fused to give a bi-nucleolate structure.

Figure 8 illustrates a case of fertilization in a compatibly pollinated ovary. A pollen-tube is seen entering the micropyle formed by the outer integument on one side and the inner integument on the other. A vesicle is evident at the tip of the embryo-sac from which, possibly, the discharge of gametes took place. Male and female gametes are seen in contact.

From sections prepared from ovaries fixed 30 hours after pollination it is impossible to say whether the ovary was compatibly or incompatibly pollinated, so alike are the preparations.

Ovaries, 48 hours after incompatible pollination, showed considerable variation amongst themselves in the microscopic details of the embryo-sacs they contained. Some sacs were completely devoid of nuclear contents, although starch persisted; a few cases were seen where the male and female elements were still unfused, and occasionally ova were observed with two nucleoli. Some sections gave clear evidence of the triple structure of the polar nucleus, yet in other sacs the polar cytoplasm could be seen, surrounded with numerous starch grains, but without the least sign of nucleoli. Again, in some embryo-sacs the polar nucleoli stained extremely feebly.

Compatibly pollinated ovaries, on the other hand, were far more consistent than those incompatibly pollinated. There was everywhere abundant evidence of fertilization, principally provided by the three deeply staining polar nucleoli. Ova were seen with both one and two nucleoli, but no cases of delayed fusion of the gametes were observed.

In nearly all embryo-sacs, the second synergida had degenerated.

At 58 hours from pollination, the contrast between the embryo-sacs of M1 ovaries pollinated compatibly and incompatibly became well marked. But even in the selfed ovaries occasional signs of fertilization having been effected could be seen. On the whole, however, the polar nucleoli were extremely faint and not the least sign of nuclear membrane could be detected. The egg was seen, occasionally, with difficulty. There were no signs of division of the polar nucleus.

Those ovaries which had received I.C.S. 101 pollen 58 hours previously showed, occasionally, two sets of polar nuclei in the embryo-sac. (See Figure 9.) A count showed 22 percent. of the embryo-sacs examined to be in this condition, the remainder still having only one polar mass, usually with three prominent nucleoli. The eggs in these sacs were frequently seen, with difficulty.

It appears evident, therefore, that divisions of the polar nuclei are initiated 58 hours after pollination in some of the embryo-sacs which have received I.C.S. 101 gametes, but not at all in those sacs which have been fertilized by M1 gametes.

Many embryo-sacs, 72 hours after pollination with M1 pollen, appeared quite empty of nuclear material, but uncollapsed. Some still contained a little starch, with the grains in a disintegrating condition. In a few the polar cytoplasm could be distinguished, but usually without any apparent organization. About one sac in 20 (actually just under five percent.) showed any trace of division of the polar nucleus. The egg where it could be seen, appeared normal but very faint.

In cross-pollinated ovaries, on the contrary, the great majority of the embryo-sacs had nuclear contents, some 82 percent. of them showing divisions of the polar nuclei. Starch was not abundant and showed signs of breakdown. The egg, still a single, undivided structure, was frequently seen.

No self-pollinated M₁ flowers remained attached to the tree for as long as 96 hours. Material collected and fixed as soon as possible after abscission showed that only very few of the ovules had collapsed, the bulk of them preserving their form till the end. The contents of uncollapsed embryo-sacs closely resembled those of the 72 hour stage, except that now 25 percent. of them showed signs of the polar mass having undergone division. Even in fallen flowers the egg nucleus, although very hard to discern, appeared normal and intact.

Cross-pollinated M₁ flowers, still attached to the tree 96 hours after pollination, when sectioned showed abundant evidence of activity on the part of the polar nucleus. Some 90 percent. of the sacs examined had two polar nuclei, some of which carried, in one and the same embryo-sac, two and three nucleoli respectively; indeed, it seemed a remarkably constant feature for one nucleus to possess three nucleoli and the other two.

Measurements of embryo-sacs were made with the aid of a micrometer eye-piece. At least 20 sacs from each type of pollination were measured from slides of material fixed 7½ to 96 hours after pollination. Table 1 gives the dimensions of the average maximum lengths and breadths at these intervals.

Figure 10 presents these figures in graph form. Apart from small differences, both curves are identical until about 50 hours after pollination, when the M₁ self curve flattens rapidly and becomes horizontal: the curve for embryo-sac size in crosses with I.C.S. 101, however, continues to rise.

The graph in Figure 11 shows the relationship between time after pollination and the proportions of embryo-sacs which exhibit first divisions of the polar nuclei in ovaries compatibly and incompatibly pollinated. It indicates that division of the polar nucleus in incompatibly pollinated ovaries is delayed and that at the time when flowers carrying these ovaries begin to absciss (72 hours, or earlier, to 96 hours) there are only from five to 25 percent. of the ovules in an active, dividing condition.

Discussion.

Examination of the literature on incompatibility in plants reveals only one or two references which report a situation similar to that obtaining in cacao. In the great majority of reported cases of incompatibility in flowering plants it has been shown that the basis of incompatibility lies in the relation between pollen-tube and female sporophytic tissues. Investigators have reported for some species a complete inhibition of pollen

germination when pollen is applied to the stigma; or if the pollen does germinate, then the tube growth is extremely limited. Numerous cases are known where the incompatible pollen-tube is slowed down in its journey in the tissues of the style so that the flower abscisses before the tubes can reach the ovules. For some species, again, it appears that tube growth in the style is normal in incompatible pollinations, but only when the tube enters the ovary proper is its further growth arrested.

The studies reported here and in a previous paper (2) show conclusively that none of these alternative factors is operative in cacao. In this plant, pollen germination is normal in incompatible matings, the pollen-tubes grow down the style equally as fast as in compatible pollinations and finally, there is no inhibitory effect in the ovary itself. Incompatible pollen-tubes reach and fertilize the ovules as easily as compatible tubes. Sears (9) has reported a similar state of affairs to exist in *Gasteria verrucosa* and holds such incompatibility to be rare in the plant kingdom. Ledeboer and Rietsema (4) have described what might be a similar case for blackcurrants.

The failure of incompatibly pollinated cacao ovaries to set fruit must therefore have its origin in some post-fertilization process or processes. Cheesman (1) has shown that, in a normally maturing cacao fruit, the fertilized egg does not divide until at least 43 days after fertilization; it would appear, therefore, that the zygote, immediately after gametic fusion, undergoes a protracted resting period. The polar nuclei, on the other hand, undergo division very much earlier than the zygote; from Figure 12, by extrapolation of the 'compatibly pollinated' curve, it may be deduced that the very first divisions of the polar nuclei, in compatibly pollinated M₁ material, take place not later than 55 hours after pollination. Their inception, indeed, may be earlier than that, since there is no real justification for a linear extrapolation; more reasonably it might be expected that the curve for frequency of polar division would be sigmoid (as its accompanying 'incompatibly pollinated' curve shows). After passing this short threshold resting period, the polar nuclei in compatibly fertilized embryo-sacs begin their first divisions in ever increasing numbers until a maximum rate is reached, somewhere about 60 hours after pollination; the rate then falls away.

For polar nuclei in incompatibly fertilized embryo-sacs, however, a different state of affairs exists. At a time when 22 percent. of the embryo-sacs in compatibly pollinated ovaries are showing

Table 1.

DIMENSIONS OF EMBRYO-SACS OF M₁ AT INTERVALS AFTER POLLINATION.

Male parent.	7½ h.	30 h.	48 h.	58 h.	72 h.	96 h.
M ₁ ...	57 μ × 38 μ	66 × 42	85 × 49	90 × 54	91 × 57	88 × 58
I.C.S. 101 ...	60 μ × 38 μ	64 × 43	80 × 51	88 × 59	96 × 64	106 × 64



Fig. 8. M1X1.C.S. 101. 30 hours after pollination. Entry of pollen-tube ; gametes side by side. X 650.

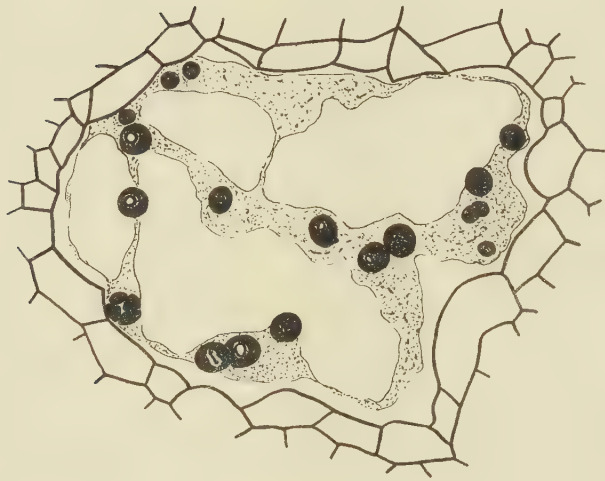
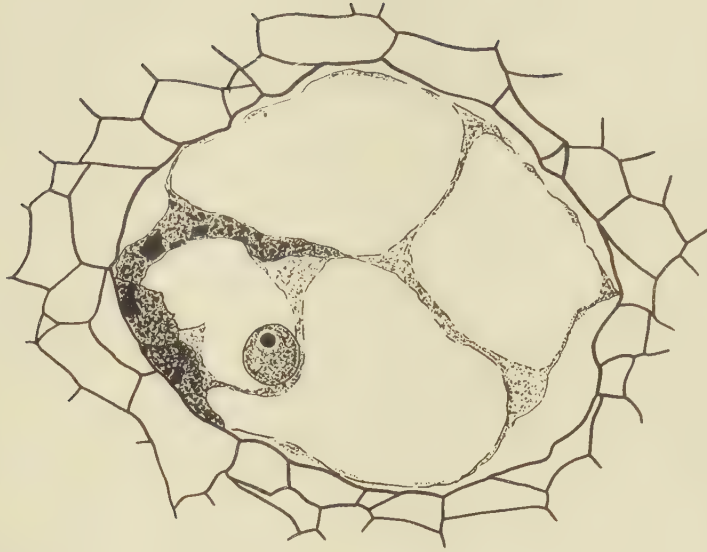


Fig. 9. M1X1.C.S. 101. 58 hours after pollination. Section on left shows two groups of three polar nucleoli ; section on right shows zygote in egg-cell. X 1300.



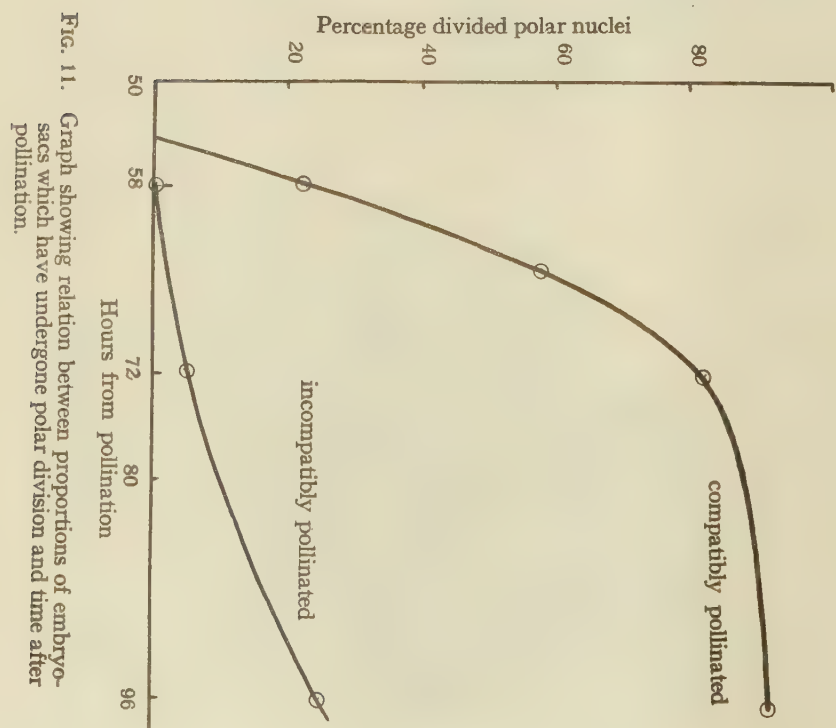
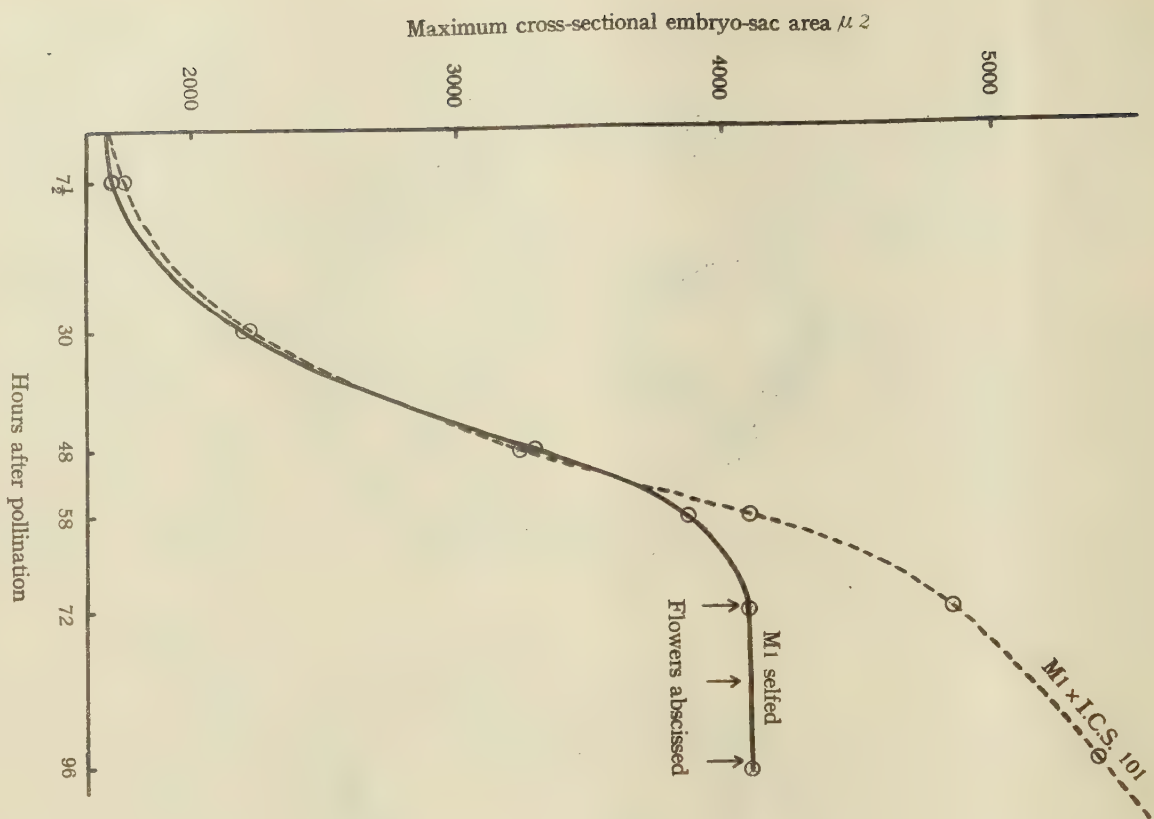


Fig. 10. Graph showing relation between embryo-sac size and time after pollination.

Fig. 11. Graph showing relation between proportions of embryo-sacs which have undergone polar division and time after pollination.

primary endosperm divisions, none can be detected in those incompatibly pollinated. With advancing time the numbers increase slowly from zero at 58 hours to 25 percent. at 96 hours, when only exceptionally can one find incompatibly pollinated flowers still attached to the tree.

It appears justifiable to state, therefore, that polar divisions in sacs which have been incompatibly fertilized are delayed and that, after this late inception, the proportion of sacs showing division increases only very slowly. Briefly stated, the whole nuclear activity of embryo-sacs in incompatibly pollinated ovaries is sluggish. In the case of nuclear fusion of the male and female gametes the same sluggishness can be detected, for even 48 hours after pollination the male and female nuclei can occasionally be seen lying side by side. This condition is never seen in compatibly pollinated ovaries at the same time after pollination.

It has been previously noted (7) (10) that ovaries incompatibly pollinated usually show some delay in, and growth before abscission. This is quite in keeping with the results recorded here; Figure 11 shows that embryo-sacs in incompatibly pollinated ovaries grow as fast after fertilization as in those compatibly pollinated, but that later a falling off in the growth rate is accompanied by flower abscission. Pound (6) has shown that abscission in cacao is probably actuated by a turgor mechanism which comes into operation within 48 hours at the very latest if the flower remains unpollinated. Incompatible pollinations appear, therefore, to exercise an influence which tends to delay, for a day or so, this turgor mechanism. This delaying influence is connected, no doubt, with activity in the ovary in general and in the embryo-sac in particular; where this activity is intense the flower may not experience abscission, but in those ovaries where, for any reason, activity is at a low level, or is late in manifesting itself, there will be only a much diminished tendency to delay or defer abscission.

Thirty hours after pollination, 80 percent. of the embryo-sacs in compatibly pollinated ovaries show signs that fertilization has been effected. At a similar interval, 64 percent. is the corresponding figure for ovaries incompatibly pollinated. Whether this difference in numbers is significant or not would, of course, require extensive studies to establish, but it must be remembered that probably in all plants a varying proportion of the ovules always remains unfertilized. Therefore in cacao it is probably not so much a question of actual proportion of ovules which receive gametes, but of the total activity in the ovary, which determines abscission, or retention, of a flower. No attempt will here be made to discuss the possible causes of nuclear 'sluggishness' in embryo-sacs which have received incompatible gametes.

To sum up, it seems likely that delayed and, slow, development of the fertilized embryo-sac is responsible for the failure of incompatibly

pollinated cacao flowers to set fruit, and for their abscission. Where, as in compatibly pollinated ovaries, activity of the contents of the embryo-sac is at a high level of intensity, then the turgor abscission mechanism does not operate.

Summary.

Evidence is brought forward, from hand and microtome sections, that pollen-tube growth and fertilization are the same in cacao ovaries pollinated incompatibly as in those compatibly pollinated.

Post-fertilization differences in embryo-sacs fertilized compatibly and incompatibly become more marked with lapse of time. Division of the polar nucleus is initiated considerably later in incompatibly fertilized embryo-sacs than in those compatibly fertilized. Moreover, the proportion of embryo-sacs showing polar divisions in the ovary which has received incompatible pollen remains always low. At abscission, incompatibly pollinated flowers show less than 25 percent. of the embryo-sacs to have undergone the primary polar division; in compatibly pollinated flowers, at the corresponding time, over 80 percent. of the sacs show nuclear division.

The fertilized egg is intact when incompatibly pollinated flowers absciss.

It is suggested that the late inception and low level of nuclear activity in incompatibly pollinated ovaries are responsible for abscission.

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(B)—CHEMICAL AND ECOLOGICAL SECTION.**THE CHEMICAL PROGRAMME OF 1939.**

By F. HARDY.

Staff.

THE biochemical work, which figured so largely in last year's report, has been continued during 1939 by Dr. H. F. Birch. Dr. E. C. Humphries, released from this work, has accordingly reopened the ecological investigations, temporarily suspended in 1935, and has begun a detailed study of the causes of cacao pod wilt. His progress is described in three papers herewith. Soil work has mainly been in the hands of Dr. E. M. Chenery (Trinidad Government Soil Chemist), with whom the College Department of Chemistry has continued to co-operate.

The Cacao Soils of Trinidad.

Investigations of the cacao soils of Trinidad have been carried out intermittently at the College since October, 1928, and numerous accounts of the work have been published during its progress. The objects of the investigations were (a) to gain information on the fertility (nutrient status) of the soils, chiefly for the purpose of advising treatments for manurial experiments; (b) to discover the chief features that distinguish "good" cacao soils (yielding naturally over 400 lb. dry cacao per acre) from "bad" (yielding less than 400 lb. ac.); (c) to describe and to delineate the distribution of different production-categories of the cacao soils in order to facilitate economic researches into the Island's cacao industry, and (d) to provide data for the comparative study of the cacao soils of other regions, such as West Africa.

These objects are essentially practical in scope, and they have involved a large amount of laboratory analytical soil work,* as well as field studies and the initiation of numerous manurial experiments. The main results have been presented in the Third, Fourth and Fifth Annual Reports for Cacao Research, especially the last-named, in which a full summary was given of the findings up to the end of 1935. So far, however, no detailed mapping of the soil types had been attempted, nor had any attempt been made finally to classify the soils, either agriculturally or pedologically.

In January 1936, the Cacao Relief Scheme was inaugurated in Trinidad, and subsequently, all manurial experiments on cacao have been under the control of the Cacao Agronomist (Dr. F. J. Pound). The results of his work have been summarised in the Sixth, Seventh, Eighth and in the present Annual Reports. In May 1936, a chemist for soil survey (Dr. E. M. Chenery) was appointed by Government, and subsequently, the soil mapping of the Island has been vigorously

conducted. An attempt has recently been made to classify the soils, and to plot their distribution by means of a Reconnaissance Soil Map and a Tentative Land Productivity Map, prepared by Dr. Chenery in May, 1939. The scheme of soil classification has been described in an article by F. Hardy, entitled "A Provisional Classification of the Soils of Trinidad", based on Dr. Chenery's typescript report; it was published in *Tropical Agriculture* for August, 1940, Vol. XVII. According to this scheme, the soils are grouped under (I) soils having free drainage (eight soil types), (II) soils having partially-impeded drainage (eight types) and (III) soils having impeded drainage (seven types). Drainage impedence may be caused by an impervious clayey topsoil, or by depressed topography, which develops perennially high water-tables. The higher productivity grades of cacao soils mostly belong to the first group.

Manurial Experiments.

A review of Dr. F. J. Pound's third year's report on the progress of his experiments is presented herewith. The soil types concerned are named and arranged according to the new Scheme of Classification mentioned in the last section; all provisional previous names of soil types should in future be discarded.

Ecological Investigations.

The three papers by Dr. E. C. Humphries forming Part II of this report, indicate the scope of the researches in the physiological ecology of cacao carried out during 1939. The problem of pod wilt was chiefly studied. The failure of cacao pods to reach maturity largely decides the magnitude of the crop, for the loss of pods through wilt may vary from 20 to over 90 per cent. of the number of pods that are set. Dr. Humphries' observations of the development and fate of each pod set on 14 cacao trees comprising part of the only mature clonal material (budded and grafted) available in Trinidad, has indicated that (at least for the season in question) a cacao tree can only bring to maturity a limited number of pods at one time. Thus, many pods that had set in June and July eventually reached maturity, but most of the pods that had set in August and September, during the time when the early crop was ripening, failed to develop, and were shed. When the early crop was ripening, however, pods that were newly set tended to stay on the tree, and thus produced a second crop. The size at which young pods wilted became smaller as the season progressed up to the time when the first crop had ripened, after which time the size at wilting increased again. These and other facts

* Some 4,800 soil samples have been analysed, involving some 52,800 separate determinations.

suggest that wilting is primarily caused by shortage of water and nutrients. The 1939 growing season was abnormally dry; the investigation is therefore being indefinitely continued, and others are contemplated, in an attempt to verify the preliminary results.

Dr. Humphries' second paper describes an investigation of the intake of water and mineral elements by the cacao pod during its development, as revealed by chemical analysis. Apparently potassium is the element most in demand, though calcium accumulates in the pod more rapidly than phosphorus.

The third paper deals with the relationship between leaf-flush and the intake of mineral elements by the shoot. It was shown by chemical analysis that the development of a leaf-flush causes a marked withdrawal of mineral elements out of mature leaves comprising a previous flush. A similar withdrawal may affect the developing pod, and may thus be a contributory cause of wilt.

Thus the ability of a cacao tree to produce a large crop of pods seems to depend largely on the presence and ready accessibility of an abundant supply of water and mineral nutrients, especially potassium. How closely this internal condition may be dependent on external environmental circumstances could only be revealed by detailed observations, measurements and chemical analyses, continued over a number of seasons for cacao trees growing under different conditions of soil and climate. It is therefore hoped that Dr. Humphries' preliminary work of last year may be continued and extended.

Cacao Biochemistry.

The nature of the work in cacao biochemistry performed during the past four years can be gauged from the titles of the papers published in these annual reports. Methods for determining catechin, tannins, theobromine and fat have been elaborated, tested and applied in attempts to trace the effects of botanical variety, degree of ripeness, environmental growth conditions, cacao fermentation and drying on the biochemical composition of the bean, and the possible relationship between composition and "quality" of commercial cacao. It cannot be claimed that much progress has been

made to these ends, many of the results being erratic and inconclusive. Among the more definite conclusions, the following may be quoted:

(a) The theobromine content of the kernel of fresh cacao beans furnishes an index of botanical type, while that of the shell of fermented cacao provides an index of the duration of the fermentation process.

(b) No significant differences have been demonstrated in the contents of tannins and fat between purple-bean Forastero and white-bean Criollo cacao varieties.

(c) No definite evidence could be obtained in support of the notion that the astringency of low-grade cacao (*e.g.* Calabacillo) is directly related to its tannin content.

(d) During ripening, increases were demonstrated in the apparent amounts of catechin and tannins in the cacao bean.

(e) During fermentation, no significant diminution in tannin content of the kernel could be demonstrated; this contradicts certain previous claims by other investigators. Similarly, but in accordance with earlier results, no change occurred in absolute fat content, though there was a relative increase in fat content, because the dry matter of the bean diminished in amount. A loss of theobromine occurred during fermentation, an observation which accords with results obtained by previous investigators.

(f) During sundrying without fermentation, the fat content of the kernel scarcely changed, but theobromine increased in amount. This last result may be accounted for by volatilisation of certain components, or by progressive decomposition of a theobromine-tannin complex.

Because the loss of astringency, said to occur during fermentation, can no longer be attributed to destruction of tannins, and since no significant differences are found in the tannin contents of beans of well-known varieties of cacao having distinctive flavours, some other component must be responsible. Certain authorities have suggested the purple pigment. Accordingly, this possibility has been examined, and the progress made in this investigation is described by Dr. Birch in the last paper of this Annual Report.

PART I.—CACAO SOIL INVESTIGATIONS.

MANURIAL EXPERIMENTS ON CACAO IN TRINIDAD:—SUMMARY OF RESULTS FOR 1939.

By F. HARDY.

(Imperial College of Tropical Agriculture, Trinidad, B.W.I.)

(I) Introductory.

THE following is a resumé of Dr. F. J. Pound's Progress Report on Manurial Experiments on Cacao for 1939 (146 typescript pages), which he submitted in March, 1940, to the Trinidad Cacao Subsidy Board. These experiments were begun in 1932 as part of the College Cacao Research Scheme; they were taken over and extended in 1936 by the Trinidad Department of Agriculture as part of the Cacao Relief Scheme. The writer is grateful to the Director of Agriculture and to Dr. Pound, Cacao Agronomist, for permission to publish this summary of main results and conclusions.

(II) Preface: A Note on the Soil Types Investigated.

The manurial experiments on cacao were taken over by Dr. Pound before the soil survey now being prosecuted by Dr. E. M. Chenery (Soil Chemist) had been started in June 1936. The names used by Dr. Pound for the cacao soil types were not those subsequently introduced by Dr. Chenery in his recent provisional scheme of soil classification*.

In the following article, Dr. Chenery's names have been given preference, though the descriptive titles adopted by Dr. Pound are mentioned synonymously. Altogether, 24 soil types have so far been differentiated in Trinidad, but not all of them are suitable to cacao growing. The more important cacao soil types may be grouped into five productivity grades, as follows:—

CACAO SOIL TYPES.

Grade I.—Very high productivity (over 500 lb. dry cacao per acre).

Montserrat Clay-Silt ("Chocolate Soil"), Montserrat Loam, *River Fine Sand* (in part), *Toco Loam* (in part), *Marper Silt* (in part), *Brasso Clay* (in part).

Grade II.—High productivity (500 to 330 lb. ac.).

River Fine Sand (in part), *Toco Loam* (in part), *Marper Silt* (in part), *Princes Town Heavy Clay*, *L'Ebranche Clay-Silt*, *Brasso Clay* (Manzanilla).

* See "A Provisional Classification of the Soils of Trinidad", by F. Hardy, *Trop. Agric.*, 1940, Vol. XVII, part 8, based on a report by Dr. E. M. Chenery describing his Reconnaissance Soil Map and Tentative Land Productivity Map of Trinidad, 1939.

Grade III.—Medium productivity (330 to 165 lb. ac.).

Sans Souci Loam, *Moruga Loam* (in part), *Sangre Grande Clay-Silt*, *Ecclesville Clay*, *Ortoire Clay* (in part).

Grade IV.—Low productivity (165 to 85 lb. ac.).

Caroni Sand (in part), *Moruga Loam* (in part), *Ortoire Clay* (in part), *Talparo Clay*.

Grade V.—Very low productivity (below 85 lb. ac.).

Caroni Sand (in part), *Maracas Sand*, *Valencia Light Sand*, *Frederick Heavy Clay*, *Piarco Light Sand*, *Nariva Heavy (swamp) Clay*, *Cocal Light Sand*.

The main features of these cacao soils whose manurial requirements have been examined (names in *italics* in the above list) are tabulated herewith; the number of experiments on each soil type is stated, and the chief manurial responses are also given in the table (p. 28)

Grade I and II cacao soils are mostly free-draining, either because of their porous sandy texture, or their open crumbly structure induced by a high lime status. Their topographical position is also suited to the maintenance of good water relationships, the soils occurring in land that is neither so hilly as to be subject to erosion, nor so low-lying as to be waterlogged. It will be noticed that their degree of acidity and their content of essential nutrients (NPK) are medium (except the two Montserrat soil types which are famed for their natural high nutrient status). These soils therefore respond in varying degree to manurial treatment, the light sandy types chiefly to potash, and the heavy clay types to phosphate manures. Unfortunately, the total area occupied by these better class cacao soils is relatively small. Excluding the least satisfactory Grade II cacao soil, Princes Town Heavy Clay, their total area, according to a recent estimate by Dr. Chenery, is probably less than 8,000 acres, which is only 0.66 percent. of the area of Trinidad, or 1.2 percent. of the area of agricultural land.

Grade III and IV cacao soils are mostly heavy, lime-deficient, very acidic clay soils, having impeded drainage, either because of their imperviousness, or because of their depressed topography. The sandy members (*Caroni Sand*, *Moruga Loam*) are highly quartzose and lack nutrient-supplying minerals, besides occupying steeply-sloping land liable to erosion. Their

MAIN FEATURES AND MANURIAL RESPONSES OF THE CHIEF CACAO SOIL TYPES.

SOIL TYPE.		Dr. Pound's Name.	Pro-duct-ivity Rating	No. of Manurial Type A (Latin Square) exp. ments.	Expts. Other Types.	(1)	(2)	(3) NUTRIENTS.			RESPONSE TO MANURES.	
Present Provisional Name.	Soil Type.					Texture.	Reac-tion.	N (Org. Matt.)	P	K	Phos-phate.	Pot-ash.
(I) FREE-DRAINAGE SOILS:												
(1) River Fine Sand ...	...	Sandy Loams ...	I, II	16	31	1, 2	3	3	3, 4	4	Oocl.	V. Def.
(2) Caroni Sand ...	...	Moruga Sands; Quartz Schist Soils	IV, V	1	12	I	4	3, 4	3, 4	3, 4	Oocl.	Oocl.
(3) Sans Souci Loam ...	...	Igneous Rock Derivatives	III	—	?	2	3	3	2	3, 4	Nil	Nil
(II) PARTIALLY IMPEDED-DRAINAGE SOILS.												
(4) Toco Loam ...	...	Calcareous Schists Soil ...	I, II	—	5	2	2, 3	2, 3	2	3	Oocl.	Dbtf.
(5) Marper Silt ...	...	Manzanilla Loams	I, II	—	3+(8)	3	2, 3	3	3	3, 4	Oocl.	Oocl.
(6) Princes Town Heavy Clay ...	...	Princes Town Black Marl	II	4	16	5	2	1, 2	4	2	Def.	Nil
(7) Moruga Loam ...	...	Moruga Silts ...	III, IV	—	?	2	3, 4	3	3, 4	2, 3	Oocl.	Dbtf.
(8) Sangre Grande Clay-Silt ...	...	Loams of S.G. Vegas; Acid Clay Alluvium ...	III	9	19	3, 4	4, 5	3, 4	4	3, 4	Oocl.	Dbtf.
(III) IMPEDED-DRAINAGE SOILS												
(9) Brasso Clay ...	...	Calcareous Clays	I, II	2	16	4, 5	2, 3	2, 3	3, 4	3	Dbtf.	Oocl.
(10) Ecclesville Clay ...	...	Brasso Clay (gypsum)	III	1	5	4	3, 4	2, 3	4	3, 4	Nil	Nil
(11) Ortoire Clay ...	...	Acid Clay Alluvium	III, IV	8	25	4	4, 5	2, 3	4	4	Nil	Nil
(12) Talparo Clay ...	...	Talparo Clay ...	IV	—	3	4, 5	5	4	3	3	Nil	Nil
TOBAGO:												
(A) (Sans Souci Loam) ...	...	King's Bay District	II	1	7	3	2, 3	2, 3	3	4	Def.	Def.
(B) (River Fine Sand) ...	...	Richmond District	I	1	8	2, 3	2, 3	2	2	3, 4	Dbtf.	Oocl.
(C) (Sans Souci Loam; River Fine Sand) ...	...	Roxborough District	I	1	9	3	2, 3	2	2, 3	4	Oocl.	Def.
Totals ...				44	167							

KEY TO INDEX NUMBERS.

(1) Texture.		(2) Reaction.		(3) Nutrients.	
DEGREE OF CLAYVINESS.	(Index of Texture.)	DEGREE OF ACIDITY.		NITROGEN (ORGANIC MATTER).	
		(pH value.)		(O.M. Content.)	
1. Sand ...	0-20	1. Alkaline ...	7.0-7.5	1. High ...	Sands, loams; Silts, clays, (per cent.)
2. Loam ...	20-30	2. Slightly acidic ...	6.5-7.0	2. Medium-high ...	(p.p.m.)
3. Silt ...	30-40	3. Acidic ...	6.0-6.5	3. Medium ...	(p.p.m.)
4. Clay ...	40-55	4. Markedly acidic ...	5.5-6.0	4. Low ...	(p.p.m.)
5. Heavy Clay ...	55-70	5. Highly acidic ...	5.0-5.5	5. Very low ...	(p.p.m.)

Note.—All values are based on laboratory determinations.

RESPONSE TO MANURES.

Def. = Definite; yield increases, over 30 per cent.
 Oocl. = Occasional; yield increases sometimes 30 per cent.

Dbtf. = Doubtful; yield increases seldom 30 per cent.
 Nil. = Never or hardly ever respond.

water and air relationships, as well as their nutrient relationships, are therefore most unsatisfactory, and may constitute the main reason why these soils generally fail to respond to manures and lime, especially in excessively wet years.

Grade V soils should never have been planted in cacao; they are either waterlogged most of the year, or are nearly devoid of nutrient-supplying minerals and lime.

(III) Summary of Dr. Pound's Main Findings and Conclusions.

(1) The most important feature of the year's results (Sept. 1938 to Aug. 1939) is the *extremely small yields* given by practically all the plots of the experiments, both control and manured. Thus, numerous instances are cited where the yields were only one-fifth to one-tenth of those of the previous year, and certain plots gave no cacao at all. This great diminution in production is attributed to *adverse weather*; the later months of 1938 were exceptionally wet, especially in the Manzanilla, Montserrat and Rio Claro Districts, where cacao fields were temporarily abandoned. The yield data thus enable a rough assessment to be made of the magnitude of the effect of excessive rainfall on cacao growing in different types of soil in Trinidad.

(2) Apart from this temporary set-back, cacao production in Trinidad has steadily declined from a peak of 75 million pounds in 1921 to 26 million pounds in 1937. Until 1930, a production of 50 to 60 million pounds would have been regarded as normal; but only twice in the last six years (1935, 44 million pounds and 1938, 42 million pounds) has this figure even been approached.

(3) Various reasons for the rapid decline in cacao yields have been advanced; for example, (i) failure to replace inefficient trees, (ii) loss of soil humus by sheet erosion, (iii) decreasing soil fertility, and (iv) increasing age and death of Immortal shade trees. With these have been associated, (v) increasing incidence of pests and diseases, (vi) less thorough cultivation and management, and (vii) lower revenue. Attempts to arrest the decline have comprised (a) better draining, (b) planting of selected high-bearing progeny, (c) manuring, and (d) orchard sanitation.

(4) Few attempts have been made to restore the environment in which cacao was originally planted after forest felling in virgin land. Dr. Pound states that "to be effective, rehabilitation must be effected on the environment as well as on the cacao tree, and whereas in the past, when cacao was young and there was ample humus, soil type appeared to be unimportant", . . . it is now admitted to be . . . "a factor of major importance". It is significant that "in its indigenous haunts, cacao is never found except on free-draining fertile loam soils of considerable depth".

(5) Dr. Pound also contends that cacao growing in Trinidad on "medium to light loams, not severely infected with Witchbroom, and already in good condition and yielding at the rate of 330 lb. per acre,* can usually be profitably manured with pen manure and certain fertilisers".

(6) Soluble phosphatic manures have proved to be more beneficial than insoluble, and soluble inorganic nitrogenous manures have proved to be not beneficial, except light dosages on humus-deficient clays. Indeed, nitrogenous artificial manures generally depress the beneficial effects of phosphates on phosphate-deficient soils. The most economic dressings of beneficial artificial manures have been found to be 300 to 600 lb. ac. (one to two lb. per tree) of superphosphate or of sulphate (or muriate) of potash, followed by smaller annual applications.

(7) Phosphate manures give only small *residual* effects, but potash manures benefit the cacao crop long after applications have been withheld. Phosphates may improve yields on potash-deficient soils, provided their potash status is first ameliorated.

(8) Manuring cannot alleviate nor mollify "adverse weather and severe disease infection", factors which were very pronounced during the season under review.

(9) There is need for further exploration of the interactions between cultivation and manuring, and great stress is laid by Dr. Pound on the value of thorough "gardening" operations and orchard sanitation, applied in conjunction with proper manuring, as means of improving cacao yields. Nevertheless, "the failure . . . to show . . . advantages from draining and round-ridging would suggest that these operations . . . are not as necessary as formerly considered to be."

(10) Pen manure is valuable for resuscitating unsatisfactory cacao on sandy soils, and for preparing the land for artificial manuring later.

(11) Liming has not proved beneficial over a period of three years, though its effects have not yet been fully explored. Losses through surface washing of ground limestone may occur on heavy loams and clays.

(12) Forking the soil around old trees likewise has given no appreciable benefit.

(IV) Manurial Responses of Different Soil Types in Trinidad.

(1) RIVER FINE SAND.

This soil type corresponds to Dr. Pound's "*Sandy Loam Soils*". It covers the valley floors and *debouchments* of the rivers of the Northern Range. No less than 16 Type A (Latin Square) experiments have been laid down on this soil type, of which 13 are at River Estate, the Government Cacao Experiment Station

* i.e. Grade I and II cacao soils. (F.H.)

situated in the Diego Martin Valley, west of Port-of-Spain. The rest are located in valleys further east, namely, Maraval, Sta. Cruz and Maracas Valleys. Nine of the River Estate experiments were described in detail in the *Seventh Annual Report* (pp. 44, 45).

(A) The *first series* of nine experiments was designed to test interactions between inorganic manures containing nitrogen, phosphorus and potassium, applied to well-managed cacao trees, aged between 20 and 30 years, growing under an annual rainfall of 65 inches. The following main conclusions were reached:—

- (a) The most effective manure is potash, either muriate or sulphate.
- (b) Potash may not be effective and may sometimes even depress the yield, (i) if applied in dosages in excess of 2 lb. per tree, (ii) if applied to soils containing more than 190 p.p.m. K_2O (chemical test), (iii) if applied for more than three years in succession.
- (c) Marked *residual effects* were shown by potash-manured plots up to at least three years after manuring was discontinued, implying that yields might later be maintained by small dosages of potash, thus reducing the costs and increasing the profits.
- (d) Liming proved ineffective in conjunction with manures.
- (e) Phosphate augmented slightly the beneficial effect of potash, but nitrogen seriously reduced it.
- (f) The natural yield of cacao growing in River Fine Sand ranges from 300 to 800 lb. per acre (average for 44 control plots, 540 lb.). Manuring with artificials raised the yield in different experiments by amounts varying from 35 to 75 percent.* the highest-yielding plot producing nearly 1,500 lb. dry cacao per acre.

(B) The *second series* of experiments was designed mainly to compare the effects of "trenching" (burying) waste vegetation and pen manure against surface spreading and forking-in, with and without inorganic manures and lime. The results described in the last two *Annual Reports* have been substantiated. The following are the main findings:—

- (a) In spite of the poor crop of the 1938-39 season, forking-in of organic manure has paid off its capital debt within three years, and given a small profit. Surface mulching with organic manure has nearly paid off its capital debt, and trenching has paid off two-thirds of its capital debt, calculated on the low value of five cents per pound of dry cacao in the field. At 7.7 cents per lb., all the organic manurial treatments would have paid for themselves in three years.

* Because of the heterogeneous nature of the tree population in Trinidad cacao fields, yield differences are regarded as significant *only when they exceed 30 percent.* (F. J. Pound, private communication).

- (b) Artificial manures (2 lb. per tree each of potassium sulphate and superphosphate) proved more effective than trenching, but the two treatments combined gave, in three years, the greatest increased yields of all (nearly 60 percent.). None of the artificial manure treatments so far has proved profitable, taking the value of cacao at five cents per pound. The treatments have been discontinued; residual effects subsequently may remove the present deficits.

- (c) Potash proved much more beneficial than phosphate when added to land that had previously been trenched.

(C) The *third series* of experiments was designed to test the effect of cane-sugar, added to raise the carbon-nitrogen ratio of the soil, in combination with potash manure and basic slag. The first year's results showed a 24 percent. increase in yield for the sugared plots, but last year's results showed only a 17 percent. increase. Potash manure gave considerable further increase, but basic slag so far has shown no additional benefit.

Note.—Other noteworthy experiments on River Fine Sand include:—

- (i) A manurial Latin Square experiment on 6-year old cacao comprising seedling progeny from six selected parent trees at River Estate, for which the first year's yield data show good but variable responses, especially for potash manure.
- (ii) A cultivation-with-manuring special demonstration at River Estate, that revealed unexpected and serious detrimental effects from draining and round-ridging which Dr. Pound considers "may be associated with the covering of the roots with soil from the drains", and "must have been doubly detrimental on the heavy acidic or marly clays in other districts". Dr. Pound suggests that "the extensive draining and round-ridging performed in the Colony in 1938 may have been very bad for the 1939 crop".
- (iii) A manurial experiment on rows of clonal cacao at River Estate (the only clonal block available), which showed remarkably variable responses, suggesting that the effect of manuring on the genetically-mixed population in an average cacao field may be extremely irregular.

(2) CARONI SAND.

This soil type, in part, is Dr. Pound's "*Moruga Sands and Silts*", occurring in south Trinidad (Moruga, Penal, Siparia), but his Moruga soil includes part of another soil type, namely, Moruga Loam. (Caroni Sand also includes Dr. Pound's "*Quartz Schist Soil*" occurring at Toco.) Only one Latin Square experiment so far has been laid down on this type occurring at Penal. The soil tested is sandy, very

acidic, and very deficient in both available phosphate and potash. The natural yield prior to the experiment was very low (100 lb. ac.). In the first year, a large (430 percent.) increase was given by potash manure, and still larger increases (660 percent.) by potash *plus* soluble phosphate manures. Adverse weather last year greatly reduced the crop, and only one picking was possible. Despite data from nine additional acre-block experiments, little exact information is available for this soil type.

The quartzose gritty-schist phase of Caroni Sand, stretching as a hilly strip westwards from Toco in the far north-east of the Island, is not considered suitable for cacao, since it is liable to be heavily attacked by Thrips when planted in this infertile sandy soil. No Latin Square experiments have so far been laid down on it.

(3) SANS SOUCI LOAM.

This, the only igneous rock soil type in Trinidad, has so far shown no response to manurial treatment. It occurs in a very rainy district in the north-east of the Island (Toco District), which is too wet for cacao, though *Hevea* rubber thrives well in this locality. It is termed "*Igneous Rock Derivatives*" in Dr. Pound's nomenclature.

(4) TOCO LOAM.

This soil type corresponds to Dr. Pound's "*Calcareous Schist Soil*", occupying a small patch of hilly country in north-east Trinidad. No Latin Square experiments have yet been laid down on it, but the results obtained from a randomised block experiment and three acre-block demonstrations conducted for two years, indicate that both potash and phosphate applications may be beneficial where deficiencies are revealed by chemical tests. The tree population is very irregular, blank pickets and poor bearers comprising some 40 percent. of the whole.

(5) MARPER SILT.

This soil type is represented in part by Dr. Pound's "*Manzanilla Loams*" said to occur at Marper Farm, but, unfortunately, the soils of Marper Farm, where numerous elaborate manurial and cultural experiments were laid down in 1930 and 1936 (See *Eighth Annual Report*, p. 32), are very mixed,* so that the results obtained give little direct information on the manurial responses of Marper Silt. The main conclusions reached by Dr. Pound from the Marper experiments briefly are: (a) Liming may be slowly beneficial in some cases. (b) Potash may be beneficial. (c) Superphosphate alone varies in its effects;

* The soil survey of the Marper area, conducted by Dr. E. M. Chenery subsequent to the initiation of the experiments, revealed the occurrence of five distinct soil types, namely, L'Ebranche (alluvial) Clay-Silt, Brasso Clay, Marper Silt and Talparo (red) Clay. Sporadic sandstone and limestone outcrops further complicate the soil pattern of Marper Farm. (F.H.)

in some cases it produces bigger responses than potash manure, in other cases, less; the two together produce less response than the sum of their individual effects. (d) Nitrogen alone depresses yields and reduces the beneficial effects of potash and phosphate. (e) Witchbroom disease is occasionally the limiting factor for yield, but it may be controlled, and yields maintained at 500 lb. ac. in years of normal weather.

(6) PRINCES TOWN HEAVY CLAY.

This has previously been called "*Princes Town Black Marl Soil*". Its manurial responses have been described in the *Seventh Annual Report*, pp. 45-46. It has responded profitably to superphosphate manure, which has given an average yearly increase over three years of 36 percent. for soil yielding naturally about 470 lb. ac. Last year, which was extremely wet* in the Rio Claro District, yields fell to nearly negligible quantities. Thus the control plots yielded only 56 lb. ac., but the superphosphate plots gave 111 lb. ac. Three other forms of phosphatic manures gave much smaller responses than superphosphate.

A special gypsum experiment again demonstrated the depressing effect of this substance (commonly present in certain clay soils of Trinidad) on the enhanced yields given by phosphatic manures. Potash manure again produced no additional yield.

A new experiment, designed to compare dressings of superphosphate of amounts one, two and three lb. per tree, showed in its first year's results the superiority of the two lb. per tree dosage.

(7) MORUGA LOAM.

This soil type includes "*Moruga Silt Soils*", grouped by Dr. Pound with "*Moruga Sand Soils*" (Caroni Sand). Certain manurial experiments conducted on this fine-grained soil type, having medium nutrient status, showed beneficial effects from phosphate and potash manures in fields where the subsoil is relatively free-draining, and where the cacao trees had been well tended, but a greater number of experiments is needed in order to determine its exact manurial requirements.

(8) SANGRE GRANDE CLAY-SILT.

This soil type, formerly called "*Sangre Grande Alluvial Soil*", mainly occupies a basin, four by six miles in dimensions; it is included in Dr. Pound's "*Loams of the Sangre Grande-Oropouche Vegas*", and probably in part in his "*Acid Clay Alluvium*". It has seriously deteriorated, largely, it is thought, through sheet

* Certain Rio Claro cacao estates were temporarily abandoned because of the disastrous effects of the abnormally high rainfall during the setting season of 1938. In most estates, whole fields or sections produced so little cacao that the crop was not worth picking. Even on certain experimental manured plots no crop was recorded. Witchbroom disease was prevalent, but not sufficiently intense to account for the greatly diminished yields.

erosion. The soil is highly acidic, nutrient-deficient and lacking organic matter; it is liable to flooding. Thrips cause serious damage in the centre of the area, and shade trees are dying from a fungous disease.

The nine Latin Square experiments laid down on this soil type (See *Eighth Annual Report*, p. 29) have given erratic and disappointing results. In some cases, pronounced benefit has resulted from phosphate and potash manures, and slight benefit from lime. An experiment in which draining, roundridging, spraying, and manuring with pen manure and artificials were compared, both singly and combined, gave large responses in the first year, but lesser responses in the second year. Single treatments were not so effective as combined treatments, especially those involving manuring with either organic or phosphate plus potash artificial manures or lime. The unsatisfactory results are attributed by Dr. Pound to an ill-adapted environment and to an unsuitable genetic strain of cacao.

Yields in recent years have diminished markedly in the Sangre Grande area, and certain experimental plots last year gave so little cacao as not to be worth recording. Nevertheless, isolated trees carrying heavy crops were outstanding, perhaps one tree in 500. Clones have been produced from some of these trees in order to test their possible resistance to the adverse conditions.

(9) BRASSO CLAY.

This soil type coincides with Dr. Pound's "*Calcareous Clays*". It is an olive-ochre coloured, stiff clay, whose top layer is not very pervious to water. Nutrient status is variable and frequently inadequate. Gypsum is typically absent (contrast Ecclesville Clay). The soil is developed from calcareous Oligo-Miocene clays of varying calcium carbonate content. Some phases are quite productive. Compared with the free-draining Princes Town Heavy Clay (derived from marlstone), into which it merges, Brasso Clay needs careful cultivation in order to produce improved yields when manured. Three years' results from two Latin Square experiments showed that both potash and phosphate manures may be beneficial; at first increased yields accrued from the phosphate applications, but later, potash became more effective, and eventually may produce considerable residual effects. The abnormal rains at the end of 1938 are considered to have been responsible for the greatly diminished yields of the 1938-39 crop. Pen manure proved more beneficial on Brasso Clay than on Princes Town Heavy Clay, but the general low level of the yields obtained indicate that, when the value of cacao is small, no profit can be expected from the simple manuring of the majority of the Brasso Clay cacao fields.

(10) ECCLESVILLE CLAY.

This soil type includes Dr. Pound's "*Brasso Clay (gypseous phase)*," and is so called in the *Eighth Annual Report*, pp. 31, 32. It was formerly

termed "Bad Brasso Clay", and its reputation for poor yields has been attributed to the toxic effect of gypsum which may occur near to the soil surface. Many areas of Ecclesville Clay have suffered severe land-slip the prevalence of which appears to be associated with the occurrence of gypsum in the parent material of the soil. Cacao planted on land-slip soil fails to thrive; the truncated profile lacks the nutrient-rich humic surface layer from which cacao derives most of its mineral nutrients and nitrogen. The available soil is compact and impervious, and its water and air relations are probably very unsatisfactory.

A Latin Square experiment designed to test the effects of potash and of sulphate-free phosphate manures on Ecclesville Clay gave inconclusive results for the first two years, and last year no crop was set. Attempts to improve the soil by applications of cane-sugar, of lime and of manganese sulphate, were all unsuccessful.

(11) ORTOIRE CLAY.

This soil type for the most part coincides with Dr. Pound's "*Acid Clay Alluvium*". It is a stiff, non-calcareous clay, having greatly impeded drainage. It is liable to flooding during rains, and cracks badly in dry weather. The soil occupies river vegas in all parts of Trinidad, and is typically developed at Guapo. It is distinguished from Sangre Grande Clay-Silt by its *red-mottled* subsoil.

Altogether, eight Latin Square experiments have been laid down on this soil type, the first in 1932, at Las Hermanas Estate,* San Rafael, near the site of the original "bad" ecological observation plot. Phosphate is the chief deficient nutrient, but very erratic results have been obtained in experiments designed to test the efficacy of phosphate manures of different kinds. In some cases, significant responses have been obtained, but residual effects have lasted only two years. The lowest dosages (one or two lb. per tree) have given the most economical results, but rarely were the treatments profitable while the value of cacao was low. Potash manure has not given any beneficial effects. The natural yield of the Ortoire Clay is only about 200 lb. ac., and no treatment has yet raised it much above 500 lb. ac. At one time, say 20 years ago, yields are said to have been much higher on this soil type. Evidently the soil has seriously deteriorated in recent years, and it has been suggested that the chief cause is loss of structure. Accordingly, and because the soil is highly acidic and lime-deficient, a thorough trial of lime as soil ameliorant has been made. In a Latin Square liming

* The *Las Hermanas* soil does not show the reddish subsoil mottling characteristic of the Ortoire Clay soil type; it resembles rather Sangre Grande Clay-Silt. The nutrient status and agricultural features of these two heavy alluvial soils are sufficiently similar, however, to warrant the inclusion of the manurial experiments under the head of Dr. Cheney's equivalent of "*Acid Clay Alluvium*", which is Ortoire Clay. (F.H.)

experiment with three different dosages of ground limestone, no very marked benefit was noted, however, possibly because lime may have been washed off the beds. It is therefore recommended that liming should be accompanied by round-ridging or by light forking in order to bury the lime, or that calcium sucrate, made from slaked lime and molasses, be used in preference to ground limestone.

Dr. Pound writes, "The low average yields and the lack of striking response* from fertilisers suggests some limiting factor, as yet undetermined, which was not a limiting factor 20 years ago. Seasons have not changed to any degree, and the only changes in environment which can be recognised are a lowering of the nutrient status, loss of humus, and ageing of the trees. The experiments show that little can be gained by correcting the nutrient status, and so efforts must now be made in other directions".

(12) TALPARO CLAY.

This "*Red-weathering Clay*" soil type has been used for cacao growing in a few places only; it is not a satisfactory cacao soil, and is classed as a Grade IV (or even Grade V) soil. No Latin Square experiment has been laid down on it.

(V) Manurial Experiments on Tobago Soils.

Dr. Pound has classed his manurial experiments in Tobago into three categories, namely:—

- (A) "King's Bay and northwards; loams and gravels and Northern Range detritals",
- (B) "Richmond; alluvial loams",
- (C) "Roxborough; peasants' properties; mostly loams".

Presumably, the first and the last categories include hilly-land soils derived mainly from epidiorite or andesite igneous rock (King's Bay); the second and the last include some very fertile cacao soil of flat vega lands.† Pending the completion of a soil survey of Tobago, this classification must provisionally be accepted.

(A) A Latin Square experiment carried on for three years in the King's Bay district, has shown that it is profitable to apply potash and phosphate manures to Tobago cacao soils which are known from chemical tests to be deficient in these nutrients, especially potash. Initial dosages of two lb. of potash and one lb. of phosphate manure per tree are recommended, but it is suggested that the yields may be maintained by subsequent smaller doses, say one-quarter of these amounts.

* In three Latin Square experiments on typical Ortoire Clay at Guapo, near Point Fortin in the Cedros Peninsula, treatments with various sorts of phosphatic manures with and without forking, gave absolutely no response. It is believed that inadequate drainage or absence of *mycorrhiza* may be responsible for this failure.

† See Cacao Soils of Tobago; No. III. Studies in West Indian Soils, F. Hardy, C. G. Akhurst and G. Griffith, 1931. I.C.T.A. pp. 1-23.

The average natural yielding capacity of the King's Bay soil during the experimental period was about 350 lb. ac., but it was raised more than 50 percent. by appropriate manuring.

(B) A Latin Square experiment, carried out for three years on Richmond Alluvial Loam, showed lesser response to potash than the last, and negligible response to phosphate and to lime; in no case was any treatment profitable. The average yield of the soil was about 580 lb. ac., but appropriate manuring raised it no more than 20 percent.

(C) A Latin Square 3-year experiment on alluvial soil at Roxborough showed only small response from trenching with coconut husks, but husks dressed with potash manure in the trenches gave a 24 percent. increase over an average natural yield of about 680 lb. ac., while phosphate augmented the yield to a lesser extent (14 percent.). Certain acre-block demonstrations gave some remarkable though variable results. In one experiment on hilly-land soil derived from igneous rock, a control plot is stated to have yielded, in 1937-38, 1,936 lb. dry cacao per acre, while a corresponding plot treated with potash and phosphate manures gave 2,088 lb. per ac., which must be a record yield for the Colony, representing a possible maximum production.* Good husbandry is considered to have been an important factor in producing these phenomenal yields.

(VI) Special Experiments.

Dr. Pound writes "It is evident that a strict enquiry must be made into the reason for and the financial implications of the cultural operations now in practice" (in cacao estates in Trinidad and Tobago), "with a view to modifying them in the light of new knowledge gleaned from experiments". Further, he writes "It is difficult to prove that many of the cultural operations performed in cocoa are directly advantageous" . . . "The experiments of the future will tend more and more to analyse the relationship between cultural and manurial treatments".

In support of these statements, Dr. Pound describes certain cultural experiments, as follows:—

(A) Shade and Drainage.

The inter-relationship between these two conditions was examined experimentally during the period 1930 to 1938 at Marper Farm. The results were presented in the *Eighth Annual Report*, pp. 33-34, from which Dr. Pound concludes that "there is no advantage in providing drains with 80 percent. shade, but a necessity for drains with 33 percent. shade. With drains at 26 ft. apart, 80 percent. shade is not needed, but, with no drains, there is a definite advantage in having 80 percent. shade". Shade-trees and drains are therefore considered to be complementary;

* See The Maximum Yield of Cacao. F. Hardy, *Trop. Agric.*, 1939, Vol. XVI, No. 8, pp. 179-191.

shade-trees may function as "overhead drains". There is less need for shade trees in sheltered valleys than on exposed ridges. Removal of half the shade trees in a block of cacao at River Estate resulted after 30 years in a 40 percent. increase in yield over that of a block left fully shaded and one entirely unshaded.

(B) *Distance Spacing.*

A spacing experiment, initiated at River Estate in 1912 and extended in 1920, has shown that the yield per acre of cacao has not been affected by the distance apart of the trees (varying from six by six feet to 18 by 18 feet), though individual tree yields have varied directly with the spacing. Below ten by ten feet spacing, no overhead shade is needed and none has been provided. Costs of cacao production have been greatly reduced in the closely-spaced blocks owing to the suppression of weed growth under the closer canopy. Witchbroom infection on the unshaded plots has been

conspicuously less than on neighbouring shaded blocks. Spacing at eight by eight feet is favoured for the conditions obtaining at River Estate.

(C) *Pruning.*

This operation as practised in Trinidad consists in removing chupons, laterals and the ends of interlaced branches; it usually results in rapid enlargement and the development of thin tufted, tortuous branches. A new method of treating neglected trees, somewhat drastic in its thoroughness, was introduced by the late Dr. Briton-Jones, and is believed to offer a means, not only of reducing size, but of "bringing vitality back to the tree", and creating a more efficient branch-system. Provided the potash status of the soil is high, the procedure soon gives rise to a thick new leaf canopy, but experiments at River Estate so far have demonstrated no significant increase in yield other than what may be accounted for by the effect of potash.

PART II.—CACAO ECOLOGY.

STUDIES IN THE PHYSIOLOGY OF *THEOBROMA CACAO*, WITH SPECIAL REFERENCE TO CHERELLE WILT.

I. PRELIMINARY INVESTIGATION OF THE FACTORS CONCERNED IN WILT.

BY E. C. HUMPHRIES.

(1) *Introduction.*

In many fruit-trees, a certain percentage of the crop fails to attain maturity. Some of the fruits are lost through disease, while the development of others ceases for no very obvious reason. This cessation in growth, or wilting, in some cases is accompanied by chemical and anatomical changes, followed by shedding, whereas, in other cases (cacao being an example), the young fruits wilt and dry up without necessarily being shed. The causes of wilting and shedding have been the subject of many investigations on various kinds of fruit with the object of controlling it and so increasing the size of the crop. There can be little doubt that there are many different processes involved in the premature fall of different kinds of fruit, but in general, fruit-shedding is believed to arise from the operation of some adverse physiological factor or factors, internal or external, in particular, water-supply and nutrition. The literature on the subject is very extensive, and no attempt will be made here to review it.

In the case of the cacao tree in Trinidad, it is a common sight to see withered young pods (cherelles) of various sizes still attached to the trees, and the failure of these cherelles to reach maturity may be presumed to constitute an important factor determining the magnitude of the final crop. Pyke (13) found that cherelle

wilt in Trinidad may account for losses of 19 to 92.5 percent. of the number of pods set by different trees, while Hewison and Ababio (5) found losses in the Gold Coast from 22 to 84 per cent.

Previous observations on the causes of cherelle wilt have usually led to a study of some related subject such as the factors governing fruit-setting; they have illustrated the great complexity of the problem without permitting the final formulation of any completely satisfactory explanation of all the physiological phenomena involved therein.

(2) *Experimental.*

FIELD PROCEDURE.

Rather than follow narrow lines of enquiry at the onset, it was decided to attack the problem of the causes of cacao cherelle wilt in Trinidad in a general manner in order to gain information concerning its nature, and to provide a basis on which future experiments could be planned.

In planning any investigations on cacao, one is confronted with the heterogeneous genetical constitution of the trees on the average cacao estate, and in the case of the present problem, it was desirable to eliminate this factor as much as possible. Consequently, recourse was made to the only mature clonal material available in Trinidad. This consisted of budded and grafted material in the "colour experiment" at River

Estate. Unfortunately the history of the stocks is not known, and only the scions are clonal. The particular trees chosen were from Parent 1304 (River Estate number), and bear long, yellow, furrowed pods. 15 trees were chosen for study in three rows of five trees, the rows being separated from one another by several rows of other varieties. The first row (Trees 31 to 35) consisted of trees budded at stake, and the second row (Trees 318 to 322), of trees budded in the nursery. No. 318 was not used in the experiment as it was a young supply. The last row (Trees 892 to 896) consisted of grafted trees. All the trees are now 26 years old. The following is a brief description of the individual trees:—

Tree 31.—Bordering on the road. Three practically equal branches fork from just above ground level. Some of the end branches are rather bare, possibly because of Thrips damage.

Tree 32.—A larger tree than 31. Main trunk jorquettes* at 6 ft. above the ground into four branches, and a chupon† arising from just above ground level jorquettes at 3 ft. into three branches.

Tree 33.—Three branches almost at ground level. Small but vigorous.

Tree 34.—Branches at 2 ft. from ground into four, and each branch subsequently divides two or three times, giving a low-spreading tree.

Tree 35.—Jorquettes 2½ ft. from ground into three branches. A big tree, with not much branching.

Tree 319.—Main branch jorquettes at 2 ft. into four thin branches. A chupon arising just behind the fork attains a height of about 7 ft., and branches into three. This tree has numerous thin branches.

Tree 320.—A sturdy tree which jorquettes at 7 ft. with a chupon growing up just behind the jorquette.

Tree 321.—Branches 6 inches from ground level into five stems which branch repeatedly, giving a straggly tree having many thin and apparently useless branches.

Tree 322.—A fairly vigorous tree branching into three branches just above the ground.

Tree 892.—A sickly tree, near the road in a rather exposed position. Jorquettes 1 ft. from the ground.

Tree 893.—Jorquettes at 1 ft. into four. Straggly.

Tree 894.—Branches at 1 ft. into two thick branches which bear the majority of the fruit.

Tree 895.—Branches near the ground and spreads.

Tree 896.—Jorquettes at 3 ft. into two equal thick branches, each of which branches again at 6 ft. A very healthy tree, well drained by a neighbouring Immortel. The largest tree of all.

All these trees have received fertiliser at the rate of 2 lb. potassium sulphate and 2 lb. superphosphate each year from 1936 to 1938; no fertilisers have since been applied.*

In the present investigation, it was decided to make detailed observations on these 14 trees in order to obtain evidence concerning—

- (1) whether there is a critical stage during the season at which the pods wilt,
- (2) whether wilting is a function of position on the tree,
- (3) how far wilting is related to external conditions.

The trees were visited weekly from March, 1939, onwards, and each new fruit was marked with an aluminium label bearing its number. The following measurements were made:—

- (1) length of the pod,
- (2) diameter of the pod,
- (3) distance of the pod from base of main trunk measured along the branch,
- (4) diameter of the branch at the point where the pod is attached.

The particular branch on which the pod was situated was also noted. Besides marking new pods, measurements of surviving pods were made and their condition noted. Each wilted pod was collected and taken to the laboratory so that it could be examined if necessary. In this way it was possible to keep a complete record of the history of each pod on the tree throughout a complete season. When setting became intense, measurements of the diameter of the pods were discontinued, but sufficient data had already

* The soil in which the three rows of trees is growing was sampled around each tree for purposes of laboratory analysis. The results (not here reproduced) showed it to be a fairly uniform fine sandy loam (Index of Texture 23), only slightly acidic in reaction (pH 6.6), fairly well supplied with organic matter (2.8 per cent.) having medium carbon-nitrogen ratio (9.0), and containing a medium-high supply of available phosphate (avail. P_2O_5 , 65 p.p.m.), but only a medium-low supply of available potash (avail. K_2O , 91 p.p.m.), in spite of the manuring. The soil is thus not particularly fertile; it may be regarded as average River Estate soil. Its yielding capacity, if it were growing a commercial cacao crop with normal River Estate husbandry, is assessed by the Manager at 500 lb. dry cacao per acre.

There is not much variation between the soil of the tree-rows, considered as units, but the soil of the north-end tree of each row is apparently appreciably calcareous, through contamination with limestone road-dust, and somewhat more fertile than the rest of the soil.

* *Jorquette* is the local planting term for the three, four or five branches into which the main stem ramifies after reaching a certain height.

† *Chupon* is an erect branch, usually terminating in a jorquette, having its leaves arranged spirally.

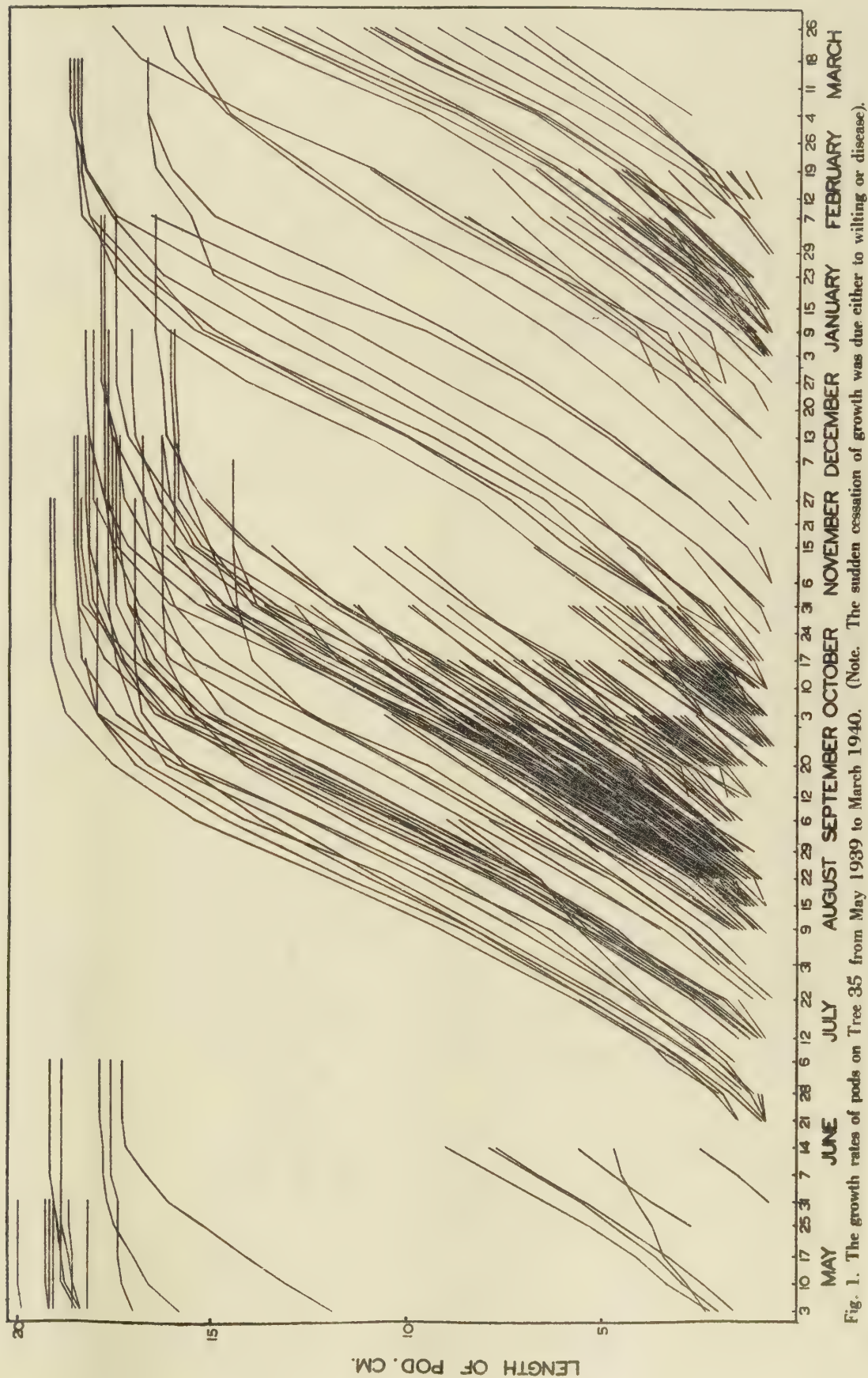


Fig. 1. The growth rates of pods on Tree 35 from May 1939 to March 1940. (Note. The sudden cessation of growth was due either to wilting or disease).

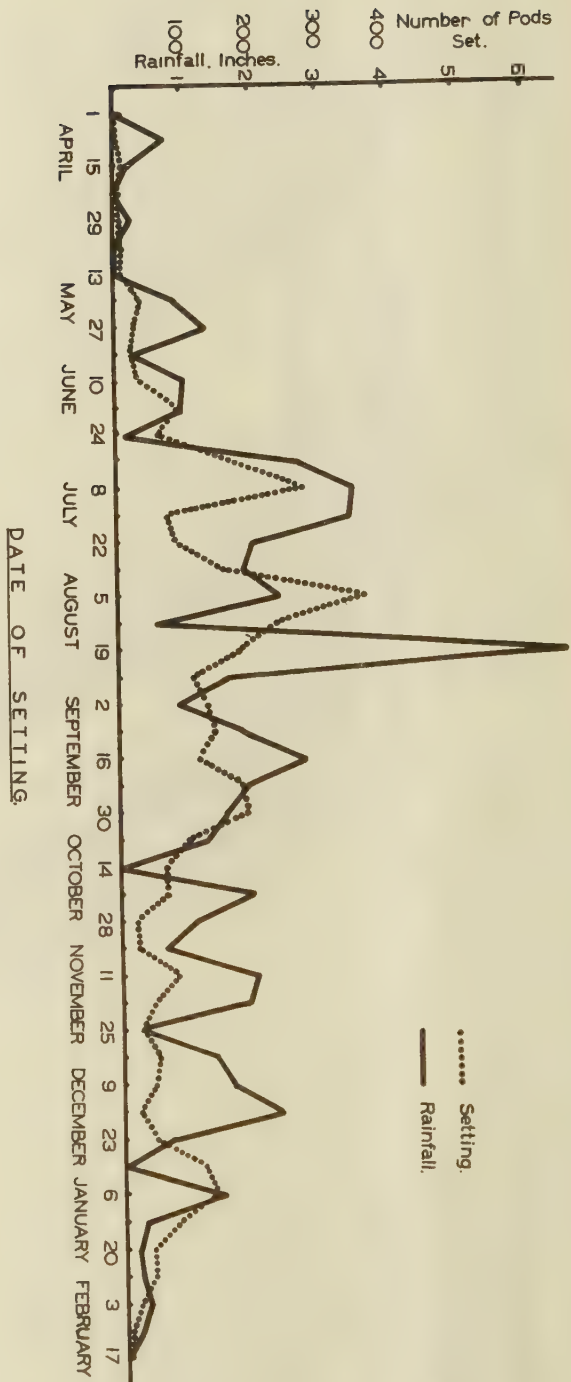


Fig. 2 The relationship between setting and rainfall.

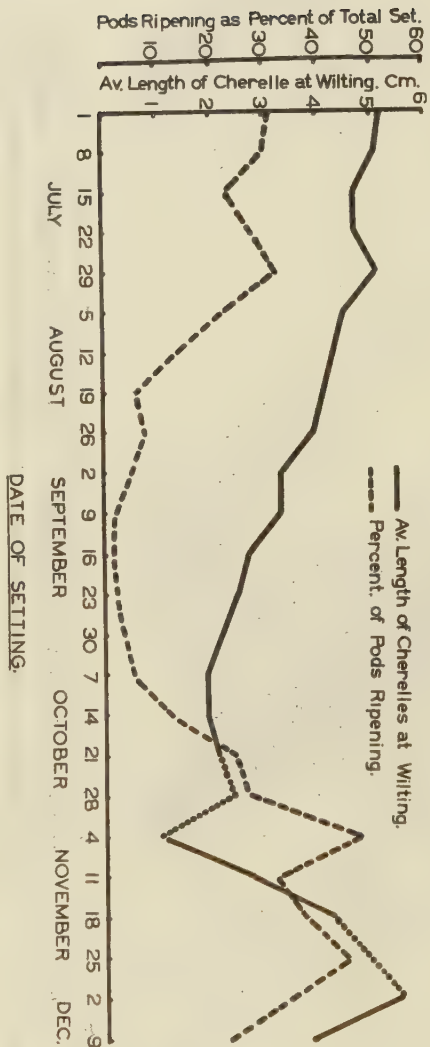


Fig. 3. The decrease in size of wilting cherelles as the season advanced. The percentage of pods set which became ripe in successive weeks is also shown.

been obtained to give an idea of the relative changes in length and diameter during the development of the particular type of pod in question. It was also found to be impossible at the height of the season to make weekly records of growth, so these were carried out about once every two weeks though each pod was examined weekly for signs of wilting.

Soon after measurements on the trees had been commenced, weekly estimations were made of soil moisture in each of the three rows of trees, samples being taken between the trees at depths of 0 to 6 inches and 6 to 12 inches. (The chief absorbing roots of cacao are considered to be confined to the surface foot layer of soil.) In September, 1939, a Stevenson Screen was established in the centre plot, and continuous records of temperature and humidity were subsequently obtained. Soil samples were taken for chemical examination at six spots round each of the 14 trees at depths of 0 to 6 inches, 6 to 12 inches and 12 to 24 inches in November (*i.e.*, at the height of the wet season), and a laboratory assessment of the nutritional status of the soil was made by means of chemical analysis.

(3) Results.

SETTING DATA.

From the length measurements of the pods, growth curves of the fruits were constructed for each tree. The case of Tree 35 is presented in Fig. 1. From these curves, it is possible to estimate approximately the date of setting (or fertilisation) of each pod by extrapolating to the origin. It is unlikely that the discrepancy of data so obtained is more than a few days, so that this estimated date of setting becomes a convenient point of reference for each pod. This is the date referred to in all cases in this paper.

A fairly accurate idea of the variations in setting may be obtained by grouping together the pods set in particular weeks. A curve constructed from the data for the 14 trees is shown in Fig. 2. This figure also shows the weekly rainfall during the same period, and it is clear

that, during this particular season, there was a general positive correlation ($r = +0.57$) between rainfall and setting. When the rainfall was high, setting was also high. It is not suggested that rain alone is responsible for bringing about setting*; the phenomenon is obviously dependent on other factors also, but rainfall is clearly important, and the response by the tree is apparently rapid. This response may be due to increased flowering through improved soil moisture conditions; it may operate through increased humidity of the air, or it may simply be due to increased water-supply to the fertilised ovules.

GROWTH OF POD.

When the growth of all the pods on a tree was followed throughout a season, some very interesting results were obtained. Reference has already been made to Fig. 1, which shows the growth for Tree 35 over a ten-month period. The growth curves of the pods on the remainder of the trees studied were similar. Very few pods began to set until the beginning of July, although there was a small burst of setting in June, possibly in response to a moderate amount of rain in that month. From July onwards, however, many pods began to grow. It can be seen from the figure that most of the pods which set in June and July reached maturity or became diseased at a relatively late stage, while pods which set at succeeding dates (August and onwards) wilted before they had attained a length of 10 cm. At the time that the crop which set in June and July was ripening on the tree (*i.e.* at the end of November and the beginning of December), many of the pods then setting grew normally and became ripe. This suggests very strongly that pods which developed early were able to obtain an adequate supply of water and nutrients, while the later pods had no supplies to draw upon, nor could the tree absorb water and nutrients at a sufficiently high rate to supply these pods; consequently wilting occurred. It is interesting to note that the new crop, *i.e.* the new fruits which will eventually come to maturity, set before the old crop was removed from the trees.

* It should particularly be noted that these results refer only to the first season's observations, when the weather at River Estate was abnormal, in that both the dry and the wet season rains were less in amount than in an average year, as the following data indicate:—

			DRY SEASON.					WET SEASON.					DRY SEASON.							
			Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.			
1939-40 ...	...	...	1.1	1.7	2.2	1.2	2.4	4.9	9.7	11.9	8.1	5.1	4.7	5.5	2.3	0.5	1.9			
Average Year†	...	...	3.5	1.8	2.0	2.0	3.8	7.0	8.9	10.3	9.4	6.9	7.9	6.7	3.5	1.7	2.0			
Dry Season, 1939...			8.6 ins.					Wet season, 1939...					49.9 ins.					Total 58.5 ins.		
Average year ...			13.1 ins.					Average year ...					57.1 ins.					Total 70.2 ins.		

† 15 years' period, 1921-1935.

SIZE AT WILTING THROUGHOUT SEASON.

It may be suggested that the primary cause of cherelle wilt is nutrient deficiency. This possibility is supported by present evidence. Careful inspection of Fig. 1 shows that, as the season progressed, the size at which cherelles wilted became smaller. This fact is brought out more clearly when the figures for each week and each tree are averaged. The results are shown in Table 1. The dates refer to the week in which the pods were set, not to the week in which they wilted. Fig. 3 shows the graph of the average figures for the 14 trees, from week ending 1st July to week ending 9th December, 1939. At the beginning of this period, the average length of the cherelles at wilting was greater than 5 cm., but during October the average length at wilting fell to 2 cm. or less. During this period, there was a much greater tendency for the wilting cherelles to drop to the ground instead of remaining attached to the trees in a dried-up condition, as they did earlier in the season. Owing to the fact that the trees were only observed at weekly intervals, it is quite possible that, during this later stage, some of the pods dropped practically as soon as they were set, and therefore were not recorded. Fruits which set in November and December, however, remained healthy for

a longer time before they wilted, and this period corresponds with the time when the old crop was ripe and the new crop was setting. In Fig. 3 has also been plotted the percentage of the pods set in any one week which eventually became ripe, and it is seen that there is a general similarity between this curve and the curve for average size at wilting. That is to say, at the beginning of the season, a higher percentage of the pods which set became ripe, while in September, less than five percent. became ripe.

Apparently at a time when pods are beginning to ripen on the tree, the chances of any pods which set becoming ripe are greatly increased (period from October onwards). It should be emphasised that the decrease in size of wilted pods was continuous throughout the period when the crop was maturing on the tree. This implies that the adverse factor or factors which caused wilting was becoming increasingly severe in the course of time, and no evidence is provided that there was a sudden appearance of an adverse factor such as a marked change in environmental conditions. As soon as the pods on the tree matured, the conditions causing wilting were ameliorated, and the size at wilting increased. Hence wilting appears to be more *immediately dependent on adverse condition inside the tree*

Table 1.

THE AVERAGE SIZE AT WILTING (IN MM.) OF CHERELLES SET IN SUCCESSIVE WEEKS ON 13 TREES, AND THE PERCENTAGE OF PODS SET WHICH BECAME RIPE.

Date of Setting (Week ending).			TREE NUMBERS.												Average. (mm.)	% of Total, Ripe.	
			31	32	33	34	35	319	320	321	322	893	894	895			896
July	1	...	52	42	60	52	—	55	—	—	35	70	40	50	66	52	31.5
	8	...	55	40	47	54	53	34	—	68	59	63	43	43	53	51	30.2
	15	...	33	54	63	57	46	33	—	—	57	35	43	46	47	47	23.1
	22	...	52	—	43	49	52	—	—	—	59	44	32	49	44	47	27.9
	29	...	81	61	49	45	68	37	—	73	39	39	33	42	49	51	32.2
Aug.	5	...	53	52	41	38	49	47	40	67	38	43	27	35	50	45	22.5
	12	...	46	38	33	35	60	39	56	68	46	46	21	27	43	43	13.9
	19	...	44	44	32	27	51	50	58	58	39	36	24	31	39	41	6.2
	26	...	36	39	47	41	59	50	49	51	26	32	23	20	28	39	7.7
Sept.	2	...	35	31	37	30	43	54	32	37	24	34	27	14	31	33	5.3
	9	...	32	30	31	29	31	30	42	42	42	32	24	33	29	33	2.1
	16	...	21	25	15	27	34	33	40	24	27	25	20	37	24	27	1.7
	23	...	21	22	23	22	30	25	32	33	34	23	10	28	20	25	2.4
	30	...	16	17	17	19	23	35	23	25	25	20	20	20	22	22	3.6
Oct.	7	...	12	19	15	18	23	19	20	18	14	17	32	18	18	19	5.4
	14	...	20	19	12	14	20	22	—	—	17	13	36	18	17	19	12.5
	21	...	22	18	16	18	19	30	12	24	12	12	44	24	16	21	24.2
	28	...	—	—	—	—	—	—	—	—	—	—	37	—	11	(24)	26.3
Nov.	4	...	—	—	11	9	—	—	—	—	—	—	—	—	—	(10)	47.1
	11	...	18	—	—	51	11	16	34	—	38	22	37	12	26	27	31.6
	18	...	—	29	—	—	—	—	—	—	58	24	30	53	56	42	37.2
	25	...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	44.7
Dec.	2	...	—	—	—	—	47	—	—	—	—	—	45	55	73	55	33.3
	9	...	27	43	—	36	53	30	31	—	—	28	30	—	64	38	22.2

than on adverse external conditions. How far the conditions inside the tree reflect an unsatisfactory environment is a matter for future investigation.

The figures for the average size of wilting of cherelles on individual trees (Table 1) also indicate, rather obscurely, that, although the trees are of clonal material, some of the trees are earlier bearers than others. In the case of Tree 894, the average size of wilting of cherelles was already only 4.0 cm. on 1st July and reached its lowest value on 23rd September, when the average size of wilting of cherelles in most other trees was still decreasing. The increase in size at wilting was correspondingly early. Further discussion of this question is not relevant at this juncture, and will be deferred for treatment elsewhere.

It must be pointed out that, since varying numbers of pods are set in successive weeks while the age at which pods wilt decreases through the season, the number of pods wilting in successive weeks becomes a variable quantity—a complex function of the two variables which in combination must initially result in an exceptionally large number of wilted cherelles in certain weeks. It is a matter of common observation in a cacao field that sometimes the number of wilted pods on the trees suddenly shows a marked increase. This sudden increase may be accounted for by the considerations outlined above; it is not necessarily due solely to a change in external environmental conditions.

From the extensive data obtained on the size at which cherelles wilt, it was found that wilting took place at all sizes up to about 10 cm. length. After they had attained this length, pods either became ripe or were attacked by fungi. Sometimes it was possible to distinguish diseased pods less than 10 cm. in length, but there can be no doubt that some pathogenically diseased pods have been included as wilted. Since the number of wilted pods was overwhelmingly preponderant, this error makes little difference to the final analysis.

RELATIVE CHANGES IN LENGTH AND DIAMETER OF POD DURING DEVELOPMENT.

As regards the limits of size between which pods wilt, it may be pointed out that the curve of diameter growth relative to length (see Fig. 4) of the particular type of pod used in this investigation (constructed from the average measurements of a large number of pods in all stages of development) shows that, until the pod was about 10 cm. in length, the diameter increased in linear proportion, but after this stage, the diameter began to increase relatively more quickly; *i.e.* the pod began to swell. Thus it appears that, at the same time that the pod reaches the limit beyond which it is incapable of wilting, certain changes are taking place in the pod which cause it to swell. This fact deserves consideration in connection with the physiological development of the pod.

ANALYSIS OF PERFORMANCE OF TREES THROUGHOUT THE SEASON.

In order to facilitate the handling of the data, the entire period has been divided into periods of eight weeks, so arranged that the beginning of the second period corresponded as closely as possible to the beginning of the period of intensive setting. The data are presented in Table 2, which shows the number of ripe, diseased and wilted pods which *SET* on each tree during these periods. The data bring out closely the fact that pods which set early in the season had the best chance of reaching maturity. The totals for the 14 trees show that, during the period 25th June to 19th August, there were 297 ripe pods, 199 diseased pods and 1,252 wilted pods, giving a wilting percentage of 76, while in the succeeding eight weeks (20th August to 14th October), the corresponding figures were 35 ripe, 17 diseased, 1,006 wilted, with a wilting percentage of 95. Table 2 also shows the percentage of wilted pods over the whole period for each tree. There is quite considerable variation, but the percentage value alone does not give much indication of the performance of the tree. Thus, Tree 892 bore the smallest number of ripe pods, but its wilting percentage was 65—the lowest of all—because of the small number of cherelles set.

EFFECT OF POSITION ON TREE.

If the ability of a fruit to survive is a question of its obtaining a continuous supply of nutrients, and particularly if the supply to the tree is not in excess of its maximum utilisable requirements, it is likely that the position of the pod on the tree will be important. This was actually found to be the case. Both the distance from the base of the main trunk (*i.e.* ground-level) along the branch to the pod, and the diameter of the branch at the point where the pod sets, were measured in the case of every fruit set on all 14 trees during the period of investigation, so that it was possible to analyse the effect of position on wilting. From the data in Table 2, it is obvious that during one period only (25th June to 19th August) were there enough data for both ripe and wilted pods to warrant a statistical analysis of the position. Such analysis is presented in Table 3. In the case of every tree, the average distance from the ground of ripe and diseased pods was *less* than the average distance from the ground of wilted pods. Similarly, the average diameter of the branch at the point at which ripe and diseased pods were borne was in all cases *greater* than the diameter of the branch at which wilted pods were borne. In all cases except those of Trees 35, 319 and 321, the difference between the distances was significant, and only in the case of Tree 321 did the differences between the branch diameter fail to be significant. The explanation of the lack of significance in these cases is not at once apparent, although the fact that the differences between the distances in the case of Trees 35 and 319 were not significant, while the differences between branch diameters were, might be explained

Table 2.
A SUMMARY OF THE PERFORMANCES OF THE 14 TREES THROUGH THE SEASON.

Tree No.	30TH APRIL—24TH JUNE.			25TH JUNE—19TH AUGUST.			20TH AUGUST—14TH OCTOBER.			15TH OCT.—9TH DEC.			Percent. wilt over whole period.
	Ripe.	Diseased.	Wilted.	Percent. Wilt.	Ripe.	Diseased.	Wilted.	Percent. Wilt.	Ripe.	Diseased.*	Wilted.	Percent. Wilt.	
31 ...	1	0	0	0	19	17	65	64	0	2	122	98	81
32 ...	2	0	8	80	26	20	78	63	2	0	99	98	77
33 ...	5	0	19	76	7	12	76	80	0	0	59	100	86
34 ...	10	0	17	46	7	10	113	87	0	1	84	99	87
35 ...	8	0	7	47	18	41	87	60	6	4	102	91	71
319 ...	1	0	9	90	19	4	54	70	0	1	59	98	83
320 ...	8	1	3	25	15	8	43	65	7	6	46	78	70
321 ...	2	2	1	20	18	17	36	51	0	0	59	100	72
322 ...	2	2	4	50	6	7	71	85	2	0	40	95	86
892 ...	0	0	2	100	1	1	13	87	4	2	11	65	65
893 ...	5	1	12	67	45	21	120	65	0	0	140	100	78
894 ...	11	3	34	71	0	0	149	100	7	1	37	82	89
895 ...	10	4	38	73	6	10	169	91	1	0	55	98	88
896 ...	18	5	20	47	20	31	178	78	6	0	93	94	74
Total ...	83	18	174	63	207	199	1,252	76	35	17	1,006	95	—

* Ripe and diseased pods grouped together for this period since, at the time of writing, it was not known how many would eventually become diseased.

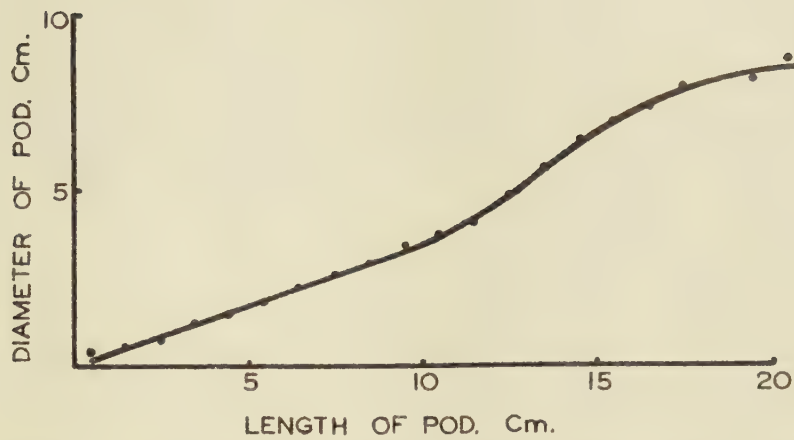


Fig. 4. The relationship between length and diameter of pod at different stages of development.

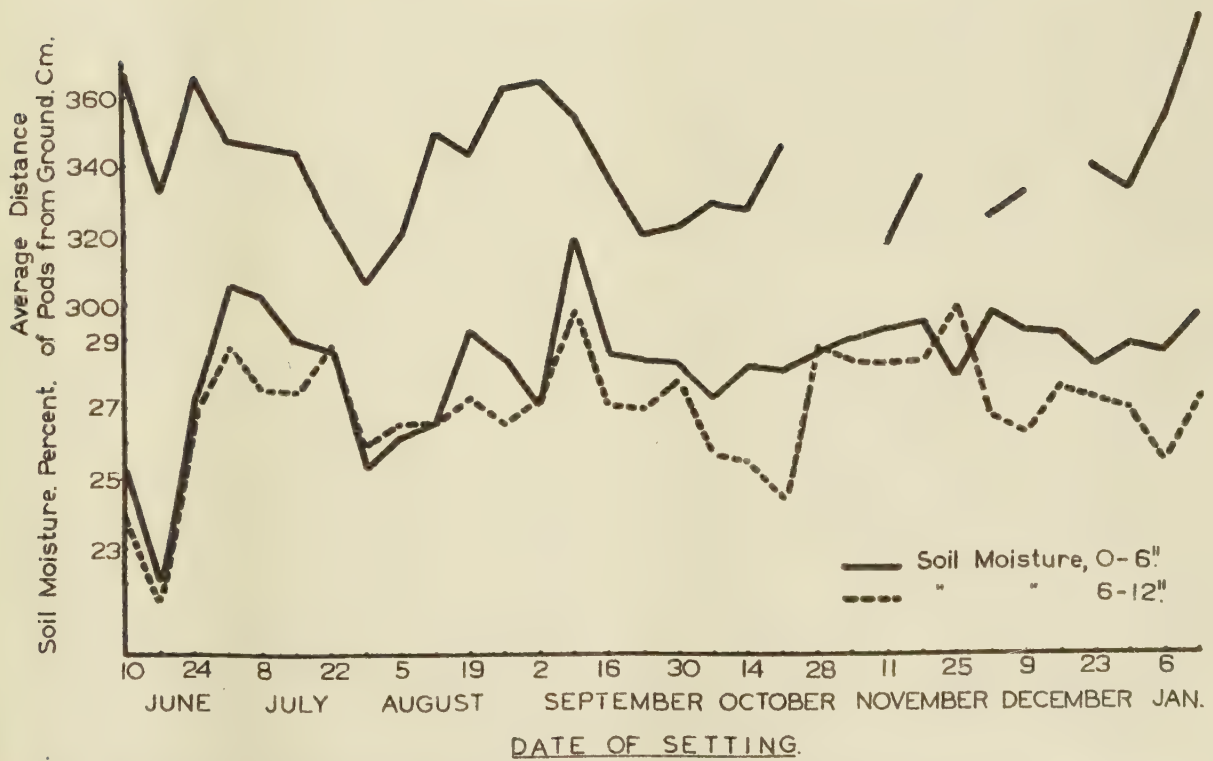


Fig. 5. Showing the average distance from the ground of pods set in successive weeks and the percent soil moisture at depths of 6 and 12 inches.

Table 3.

A COMPARISON OF THE POSITIONS ON THE TREE OF RIPE AND WILTED PODS SET DURING THE PERIOD
25TH JUNE TO 19TH AUGUST, 1939.

Tree No.	Average distance from ground of ripe and diseased pods. (cm.)	Average distance from ground of wilted pods. (cm.)	Level of Significance. (P)	Average diameter of branch at point of attachment of ripe and diseased pods. (cm.)	Average diameter of branch at point of attachment of wilted pods. (cm.)	Level of Significance. (P)
31	247	294	<0.05	4.8	2.5	<0.01
32	376	423	<0.05	3.9	1.9	<0.01
33	197	302	<0.05	4.8	2.8	<0.01
34	214	331	<0.05	5.0	2.8	<0.01
35	347	379	Not Signif.	4.4	3.0	<0.01
319	278	311	Not Signif.	5.3	3.6	<0.01
320	339	398	<0.05	5.4	3.6	<0.01
321	257	276	Not Signif.	4.8	4.1	Not Signif.
322	165	272	<0.01	5.8	4.1	<0.05
892*	(206)	(322)	—	(14.3)	(2.9)	—
893	298	361	<0.01	4.9	2.5	<0.01
894†	—	—	—	—	—	—
895	208	359	<0.01	7.0	3.1	<0.01
896	281	420	<0.05	6.8	3.0	<0.01

* Tree 892 did not produce enough ripe pods to warrant statistical analysis.

† In the case of Tree 894, all the pods set during this period wilted.

by the peculiar shape of the tree. It is clear, then, that pods which set on thinner branches and therefore further from the ground, tend to wilt more than those which set on thicker branches nearer the ground. In this connection, it may be noted that Pound (9) has shown that pods are smaller the further they are from the ground, and Esbjerg (3) has shown that higher yields are obtained from apple trees having trunks 0.5 m. long than from those having trunks 1 m. in length under similar conditions.

Once the importance of position of the pod on the tree is established, it is of interest to examine the factors which determine position on the tree throughout a season. One of these was found to be the moisture content of the soil. In Fig. 5 have been plotted the weekly percentage soil moisture values at depths of six inches and 12 inches and the average distance from the ground of pods set between the week ending 10th June and the week ending 13th January. The relevant figures are presented in Table 4. In all cases, the average for the distance from the ground was based on the measurements for more than 40 pods. The resemblance of the soil moisture curves (especially the one for the surface six inches of soil) to the distance curve is very striking, and the resemblance becomes even closer when it is remembered that the soil moisture curves are displaced slightly to the right because, although the moisture samples were taken mid-weekly, the points obtained have been plotted on the last day of the week in uniformity with the distance figures. The variance of the position on the tree for each week was also calculated, and this proved

to be a curve which had a general inverse appearance to the moisture curve, showing that, in periods of increased moisture, pods are scattered more at random over the tree. The close relationships between the percentage of moisture in the soil and the distance of the pods from the ground, suggests that the cacao trees responded very rapidly to moisture changes in the soil, which in turn suggests that the trees were constantly subject to a water deficit, and there was immediate response to improved facilities for obtaining water by pods setting higher on the trees. If however, adverse moisture conditions intervene, the more remote pods will be the first to be affected, and may consequently wilt. Thus, in addition to the nutritional deficiency theory adduced to explain cherelle wilt, it is very possible that wilting is sometimes caused by a deficient supply of water to the developing cherelle. Low water-supply will decrease the plant's ability to obtain nutrients, and *vice versa*, the two growth-factors being closely inter-related.

Since the cacao tree is very sensitive to fluctuating moisture conditions, the importance of maintaining an even moisture status at an optimum level hardly needs emphasis, and the evidence put forward here lends additional support to the claims of Hardy (4) who has stressed the importance of avoiding fluctuations in moisture content of cacao soils. Referring again to Fig. 4, it is noted that, from the beginning of June until about the middle of September (*i.e.* during the time when rainfall is fairly continuous), the moisture content of the second soil layer (from 6 inches to 12 inches depth) was much the same

Table 4.

THE AVERAGE DISTANCE FROM THE GROUND OF PODS SET IN SUCCESSIVE WEEKS, AND THE SOIL MOISTURE CONTENT AT TWO DEPTHS.

Date of Setting (Week ending).	Average distance of pod from the ground. (cm.)	SOIL MOISTURE : (PERCENT OVEN-DRY WEIGHT).	
		(0-6") Surface layer.	(6-12") Second layer.
June 10 ...	367	25.4	24.0
17 ...	333	22.1	21.5
24 ...	365	27.4	27.0
July 1 ...	348	30.5	28.8
8 ...	346	30.2	27.6
15 ...	344	29.0	27.5
22 ...	324	28.7	28.8
29 ...	307	25.3	26.0
Aug. 5 ...	320	26.2	26.6
12 ...	349	26.6	26.6
19 ...	343	29.2	27.3
26 ...	362	28.5	26.6
Sept. 2 ...	364	27.1	27.3
9 ...	354	31.9	29.8
16 ...	336	28.6	27.1
23 ...	320	28.4	27.0
30 ...	322	28.3	27.8
Oct. 7 ...	329	27.3	25.7
14 ...	327	28.2	25.5
21 ...	345	28.1	24.4
28 ...	—	28.6	28.7
Nov. 4 ...	—	29.0	28.3
11 ...	317	29.3	28.2
18 ...	336	29.5	28.3
25 ...	—	27.9	29.9
Dec. 2 ...	325	29.7	26.8
9 ...	332	29.2	26.3
16 ...	—	29.1	27.6
23 ...	339	28.2	27.3
30 ...	333	28.8	27.0
Jan. 6 ...	352	28.6	25.4
13 ...	382	29.7	27.3

as the moisture content of the surface layer. From about 16th September for the next few weeks, the rainfall gradually decreased, until in the week ending 14th October, no rain fell. Thus, in Fig. 4, it can be seen that the moisture content of the second layer diminished much more rapidly than that of the surface layer, which remained remarkably constant. Immediately following this period, the rainfall was again fairly continuous, and the moisture contents of the two layers were very similar, until about 25th November, when rain was only slight, and the moisture content of the second layer began to diminish below that of the top layer of soil. In other words, the moisture fluctuations of the second layer were greater than those of the surface layer.

(4) Discussion.

As a result of his studies of fruitfulness in cacao, Pound (10) considered that "there is no doubt "that weather conditions play a large part (in "wilting), for there seems to be always a large "amount of wilting in years when there is no fine "spell in October". The writer's data for the 1939 season seems to lend no support to this view, but it must be stressed that detailed observations over a period of years would have to be accumulated before a causal relationship between weather and wilt could finally be established. The fact that the year under observation was abnormally dry may account for the apparent independence of wilting on weather which was observed; the relative dryness may indeed have permitted the clearer analysis of the fundamental causes of wilt than would have been possible in a wetter or more "normal" year.

Other suggestions by Pound (*loc. cit.*), namely that wilting may be attributed to incomplete pollination or to incompatibility, here deserve consideration. He eliminated the former possibility by noting an insignificant difference between the numbers of ovules in wilted and sound cherelles. That incompatibility may be an important factor seemed more probable, since Rounce and Smart (14) had observed that maximum wilting occurred at the same time after setting as Cheesman (1) had indicated to be coincident with the first division of the zygote. In the present investigation, however, it was found that wilting took place in cherelles of all sizes up to 10 cm. length, and that the size at which wilting occurred varied throughout the year; *i.e.* in the particular season under observation, there appeared to be no critical size of wilting, although in the early part of the period the average age of wilting corresponded approximately with the age at which the first division of the zygote takes place.

Pound (11) has also stated that the critical age for wilting coincides with the time at which the cherelle is commencing its most rapid growth, but it will be shown later [Humphries (6)] that in the present investigation, the maximum growth (*i.e.* time of greatest increase of dry weight) of a cacao pod occurred when it had passed the stage at which, according to Pound's view, it should have wilted. Pyke (13) found that, in general, there was an increase of wilting at the time of, or immediately following, each leaf flush. No connection between flushing and wilting was observed in the present investigation, however, although it cannot be denied that a heavy flush of leaves may make a considerable demand on the nutrient reserves of the tree. In an attempt to assess these demands, an investigation has been carried out on the mineral requirements of cacao leaves [Humphries (7)]. Pyke (*loc. cit.*) also states that there appears to be no very close connection between the percentage wilting and the final crop of an individual tree. This observation was confirmed in the present investigation.

McDonald (8) in his environmental study of the cacao tree found that a short dry spell of two to three weeks' duration caused many cherelles to shrivel, and although this effect was not observed during the season of the present investigation, such a result is likely, having regard to the sensitiveness of the cacao plant to moisture fluctuations. It should be remembered, however, that the longest dry spell in a normal wet season occurs in late September or early October (the "petit carême") at a time when wilting is intense through competition with the maturing crop, so that, in order to prove whether or not a short spell causes increased wilting, close analysis of a considerable amount of data would be required. It should also be remembered that a short dry spell might have a much more severe effect on some soil types than on others. Pound (12) finally came to the conclusion that wilting of young cherelles was largely due to physiological causes, and suggested that excessive rainfall, extreme dryness, or a heavy flush of growth, may all at times be major factors inducing wilting.

The experimental results presented in this paper afford evidence that wilting is caused through some nutrient deficiency, and in some cases through water deficiency, or both, in-so-far as the two factors are linked together. It is noteworthy that Cope (2) has shown that potash manures reduced cherelle wilt on trees at River Estate, while phosphate had no such effect, an observation in accord with the discovery [Humphries (6)] that potassium is the mineral element required in largest quantities by the developing cacao fruit.

(5) Summary.

1. A preliminary investigation has been made into the factors concerned in cacao pod wilt.

2. The following evidence is adduced in support of the theory that wilt is primarily due to nutrient and water deficiency:—

(a) Pods which set early in the season reached maturity or became diseased at a relatively late stage, but pods set later usually wilted. When the crop began to ripen, many of the pods then setting did not wilt, and eventually became ripe.

(b) As the season progressed, the size at which cherelles wilted became progressively smaller until the crop was removed from the trees, after which the size at wilting became larger again.

(c) Pods on thinner branches, and therefore further from the ground, wilted more than those on the thicker branches.

(3) No evidence was found that weather had any effect on incidence of wilt, but it should be noted that the total rainfall and rainfall fluctuations were less in amount during the period of observation than they are in a more "normal" year.

(6) Acknowledgment.

The author wishes to thank Prof. F. Hardy, Dr. T. G. Mason and Dr. H. R. Barnell for continued interest and constructive criticism. He also wishes to thank Mr. R. O'Connor, Manager of River Estate, for facilities freely placed at his disposal, and Ahomet Khan and Ramnarine Maraj for carrying out the large number of measurements.

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II.—GROWTH RATE AND MINERAL INTAKE BY THE POD.

By E. C. HUMPHRIES.

(1) Introduction.

It is known that the developing fruit of the cacao tree is liable to physiological wilt, but only during the early part of its development, viz.: up to approximately 70 days after fertilisation [Humphries (2)]. After this time, losses of fruit are entirely due to pathogenic causes.

The experiments described in this paper were designed with the object of obtaining a general idea of the changes which take place during the development of the pod, with special reference to water-content changes, fresh-weight and dry-weight increments, and mineral intake.

A similar set of cacao trees growing at River Estate were employed in this investigation as were studied in the last. It should therefore be borne in mind that the results obtained refer to a particular group of trees growing in a particular environment during a somewhat abnormal season. It is hoped to continue the experiment (with suitable modifications) during one or more subsequent years.

The data so far accumulated show certain interesting features having bearing on the general question of mineral nutrition of the cacao tree, which seems to warrant their publication at this stage.

(2) Experimental.

SAMPLING METHODS.

It is necessary in an experiment of this nature to obtain truly representative samples at frequent intervals throughout the life cycle. The most straightforward method would be to tag pods at the time that they appear, and to collect samples after different periods of time. Owing to the fact that as many as 95 percent. of the pods are liable to wilt, the labour involved in marking a sufficient number of pods to give requisite samples of all ages is prohibitive, and recourse must therefore be made to an indirect method. The method actually adopted consisted in employing a size index of development instead of time, and relating this to size-time data already available. Thus, the first size-class included pods between 1.0 cm. and 1.9 cm. in length, the second 2.0 cm. to 2.9 cm., and so on, up to a size-class of 18.0 cm. to 18.9 cm. In this last class, two samples were collected, one consisting of unripe pods, and the other of pods which were just yellowing. In most cases, a sample consisted of 25 pods, but in the first four size-classes, 50 pods were collected. Care was taken that not more than one pod in a sample was picked from a tree. The average age of the pods in any class was determined by averaging the growth data obtained from a large number of pods in a previous investigation (Humphries, *loc. cit.*). Thus, the pods of the 1.0 to 1.9 cm. class were regarded as 18 days old, those of the 2.0 to 2.9 cm. class, 25 days old, and so on.

The end of October was selected as a suitable sampling time, because pods of all sizes were then present on the trees. The pods were collected and put into tins with tight fitting lids, and quickly conveyed to the laboratory, where they were weighed and sub-sampled for moisture and ash determinations. Moisture was determined by drying for 48 hours at 105°C, and the material for ash analysis was heated in an electric muffle at 500°C. Potassium was determined by the sodium-potassium-cobaltinitrite method, phosphorus by the amino-naphthol-sulphonic acid colorimetric method, calcium as oxalate, and nitrogen by the Kjeldahl method.

(3) Results.

FRESH-WEIGHT AND DRY-WEIGHT CHANGES DURING GROWTH.

The changes in fresh-weight, dry-weight and moisture content of a pod, at various ages from 18 days to the just-ripe stage at 170 days, are presented in Fig. 1, which is constructed from the data of Table I. The curve of fresh-weight has the familiar sigmoid form, and shows that, at the period of maximum growth (from about 93 to 107 days), the fresh-weight increment was 131.75 g., an average of 9.4 g. per day. The corresponding dry-weight increment over the same period was 16.44 g., or 1.17 g. per day.

Table I.

CHANGES IN FRESH-WEIGHT, DRY-WEIGHT AND WATER CONTENT OF A POD DURING ITS DEVELOPMENT.

Age of pod (Days.)	Average Fresh-weight of a pod. (g.)	Average Dry-weight of a pod. (g.)	Moisture Content per 100 g. Dry Weight. (g.)
18	0.3049	0.0311	881.5
25	0.9051	0.1349	570.0
32	2.3185	0.3362	589.4
39	4.2576	0.6131	592.6
46	8.2046	1.1322	625.7
52	14.52	2.05	607.9
57	19.47	2.67	630.1
63	32.44	4.28	660.2
68	39.24	4.75	728.8
73	53.96	6.26	761.2
78	79.84	9.18	767.6
84	102.20	11.34	804.5
87	142.44	14.53	835.5
93	177.65	20.07	787.7
99	219.45	24.14	812.8
107	309.40	36.51	750.5
122	392.40	51.40	663.8
143	459.85	70.82	549.2
170	493.44	97.30	407.4

Water content.

The water content of the youngest sample (18 days) was very high, viz.: nearly 900 g. per 100 g. dry-weight (Fig. 1). At 25 days, the water content had fallen considerably to 570 g. per 100 g. dry-weight. From this point onwards, the water content increased again to a value of over 800 g. per cent. dry-weight, when the pod was about 90 days old. The water content then began to fall steadily, until, in the ripe pod, the value was about 400 g. percent. dry-weight. The most rapid increase in moisture content was from about the 50th day (a time when it has been suggested that the first division of the fertilised ovum takes place [Cheesman (1)], until about the 87th day.

Dry-weight.

The increase in dry-weight was exponential until about the 107th day, after which the increase was practically linear. Figure 2 shows the logarithm of the dry-weight plotted against time. The curve resolves itself into two straight lines; that is, the dry-weight increase obeys a compound interest law, but the rate of accretion of dry matter alters suddenly at about 50 days. The point of inflexion in the dry-weight curve shown for this age corresponds with the time of the first division of the zygote [Cheesman (*loc cit.*)].

INTERNAL CHANGES IN THE POD DURING DEVELOPMENT.

Pods in each size-class were inspected at sampling in order to obtain a general idea of the main changes which take place during development. The more obvious changes are recorded in Fig. 1. The beans, almost microscopic at first, gradually enlarged, and, at about 60 days, were seen to contain a liquid. After 90 days, the contents of the beans had become gelatinous, and about

ten days later, they were almost solid. At this stage, the cotyledons could be clearly distinguished. In the next sample (107 days), the majority of the beans had become purple, and, at 122 days, all the beans were purple, and were still enlarging. In the ripe stage (170 days), the white pulp had broken down, except in the immediate vicinity of the beans, which were separate from one another. The most rapid changes in the bean therefore apparently take place between about 90 and 110 days, a time when the fresh-weight and dry-weight increments are greatest, and when the moisture content has reached its maximum, and is beginning to decline.

THE MINERAL CHANGES.

The data presented in Table 2 shows the percentage of total ash, total nitrogen, P_2O_5 , K_2O and CaO present in the dry matter of the pod at different stages of development. The drifts in these various components are also shown in Fig. 3. In the early stages, the *total ash* showed a slight increase up to about 50 days, that is, the uptake of minerals increased relative to the increase of dry-weight as a whole. From fifty days onwards, the percentage of total ash began to fall until the 87th day, when there was a marked increase. In the next sample, the value had fallen again, but the succeeding value was again high. Subsequently, there was a fall in the percentage ash; it then remained constant after the 122nd day until maturity. *Potassium* showed a similar trend, with marked increases in the 93rd and 107th day samples. *Nitrogen* showed a fairly rapid fall for about 50 days, then the decrease became less rapid. There are two peaks in the curve at the same dates as in the case of the other components. After that point, the decrease assumed approximately the same rate as before. In the case of *calcium*, there was

Table 2.

ASH ANALYSES OF A POD AT DIFFERENT STAGES OF DEVELOPMENT. RESULTS EXPRESSED ON BOTH DRY-WEIGHT AND ASH BASES.

Age of pod. (Days.)	TOTAL N.	ASH						
		Percent. Oven-Dry Material.				Percent. Ash.		
		Ash	P_2O_5	K_2O	CaO	P_2O_5	K_2O	CaO
32	2.48	8.52	0.89	2.92	1.52	10.45	34.27	17.83
39	2.49	8.60	0.90	2.92	1.51	10.47	33.97	17.57
46	2.37	8.70	0.83	3.03	1.54	9.53	34.83	17.70
52	2.19	9.24	0.84	3.13	1.76	9.09	33.87	19.04
57	2.18	8.82	0.80	3.05	1.67	9.06	34.59	18.93
63	2.20	8.92	0.83	3.13	1.63	9.31	35.12	18.29
68	2.12	8.48	0.77	2.86	1.54	9.07	33.70	18.16
73	2.19	8.42	0.83	3.01	1.50	9.85	35.72	17.82
78	1.98	8.03	0.77	2.76	1.43	9.58	34.34	17.80
84	2.02	7.81	0.72	2.76	1.26	9.22	35.35	16.14
87	2.20	8.93	0.82	3.30	1.39	9.18	36.95	15.59
93	1.97	7.92	0.75	2.82	1.28	9.47	35.60	16.17
99	2.18	8.74	0.63	3.24	1.23	7.21	37.07	14.08
107	1.91	7.34	0.59	2.88	0.88	8.03	39.22	11.98
122	1.80	6.63	0.64	2.76	0.73	9.64	41.62	11.01
143	1.73	6.43	0.64	2.65	0.59	9.95	41.20	9.17
170	1.64	6.67	0.65	2.75	0.48	9.74	41.20	7.19

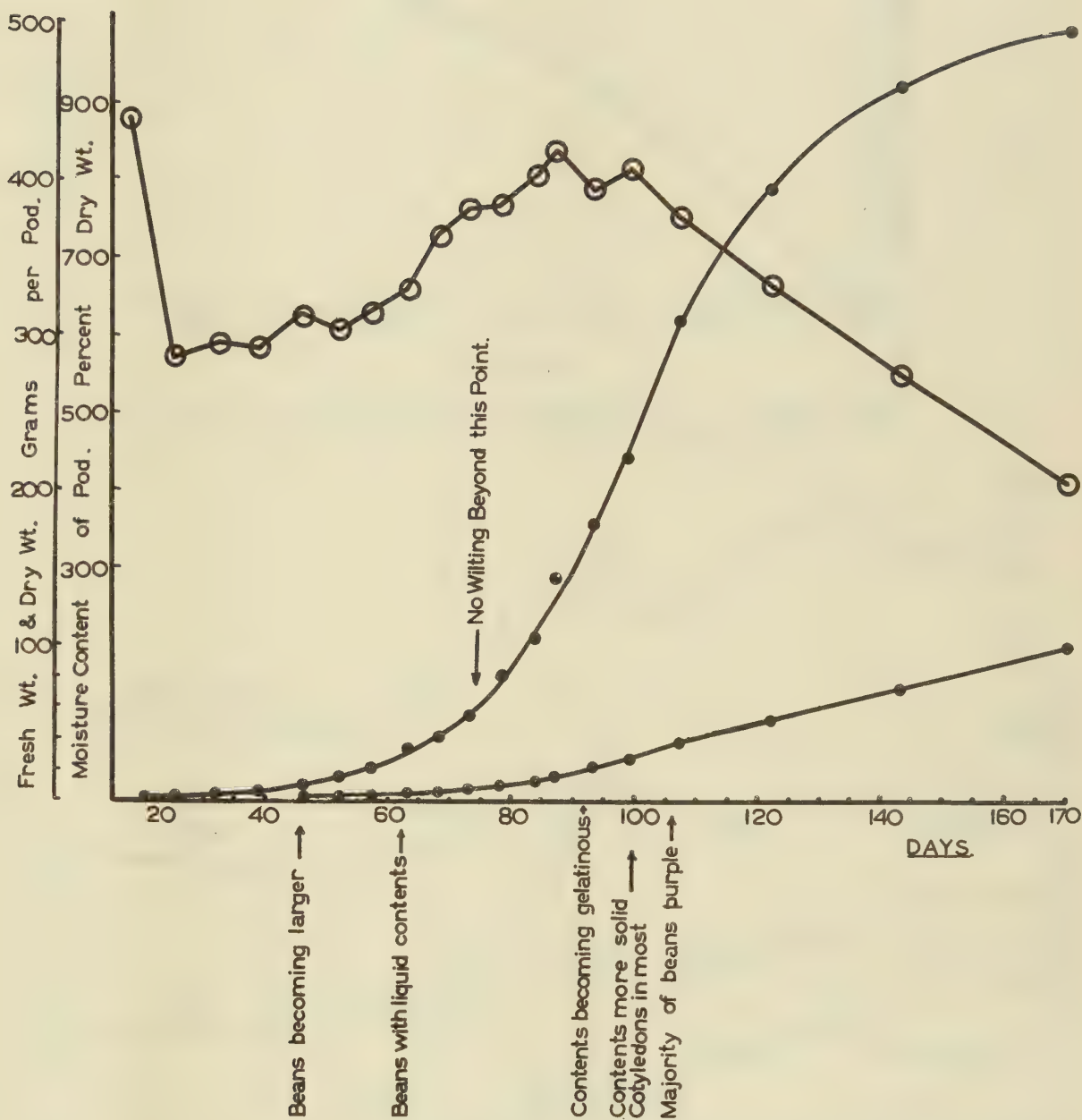


Fig. 1. The increase in fresh-weight and dry-weight of a pod during its development and changes in water content. The obvious changes occurring in the bean are also shown.

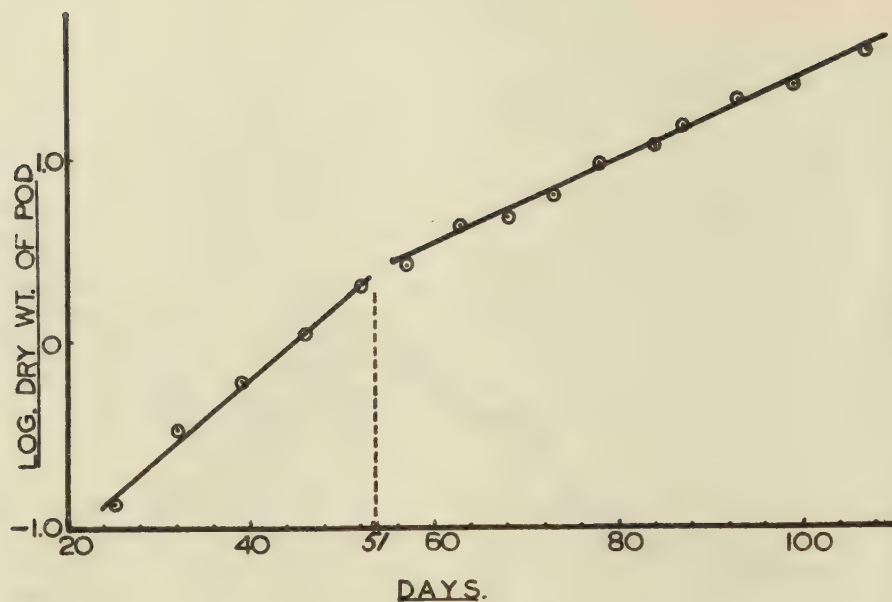


Fig. 2. Logarithm of dry-weight plotted against time. Note the inflexion in the curve occurring at about 51 days.

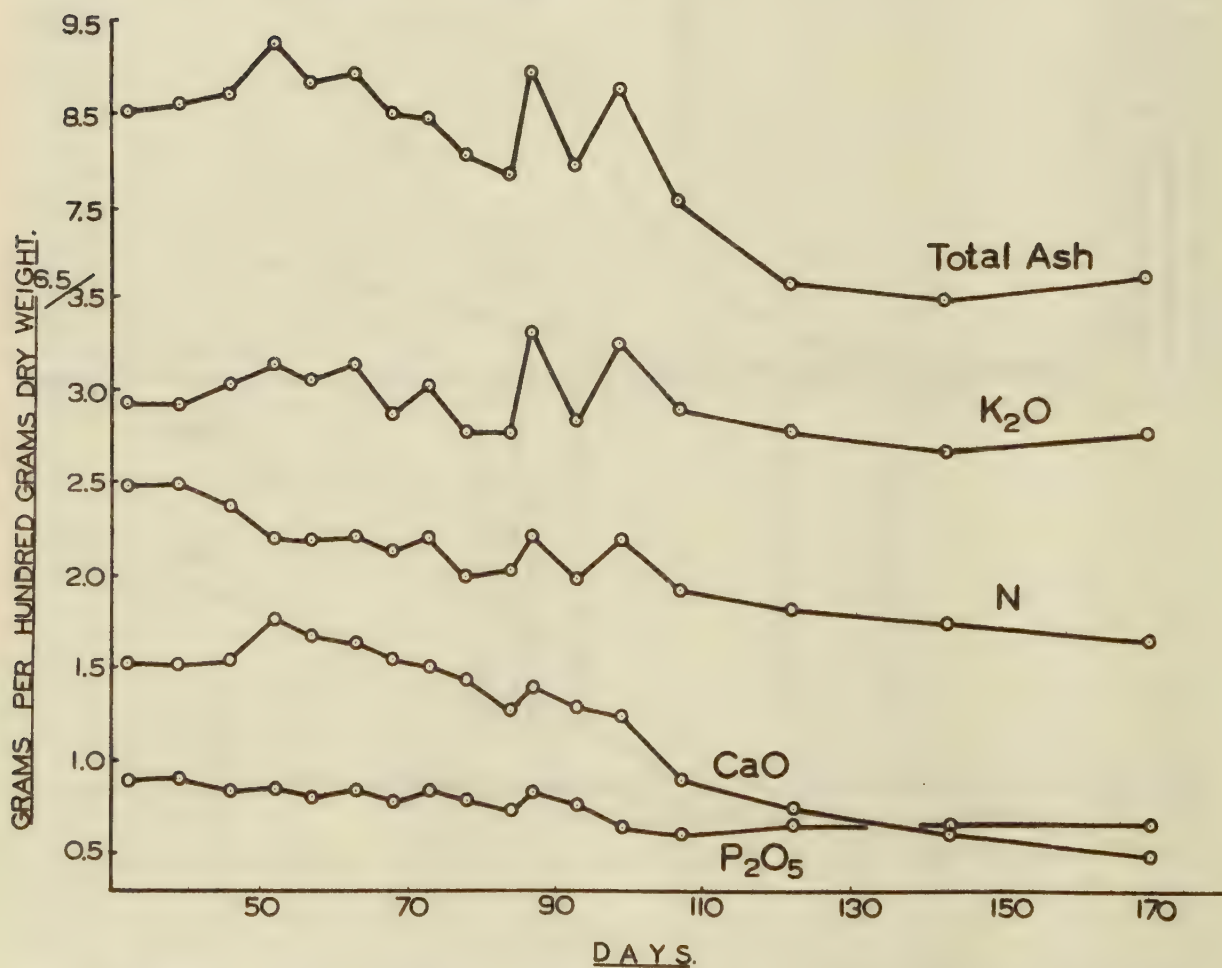


Fig. 3. The changes in total ash, total nitrogen, K_2O , P_2O_5 and CaO occurring in the pod at all stages of development. The results are expressed as grams per 100g. dry-weight.

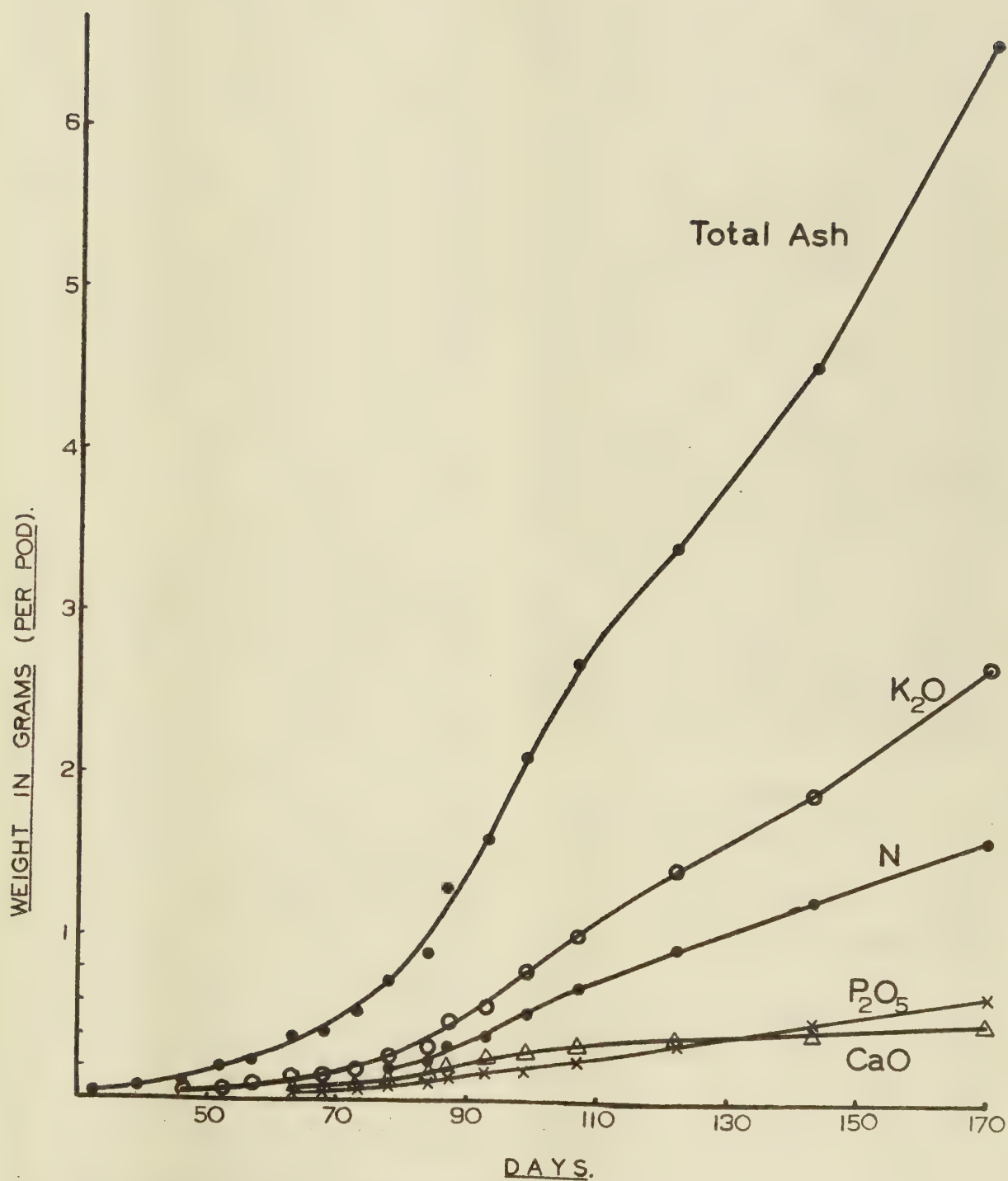


Fig. 4. Absolute changes in the ash constituents of the pod at all stages of development.

an increase until about the 50th day, and then a fall which was arrested between the 93rd and 107th days by three high values, after which there was a steady decline until maturity. *Phosphorus* was present in least amount of the elements investigated, and showed a very slight downward drift for 87 days, then a slight increase in the next two samples, after which there was a slight increase up to the 122nd day, then the values remained constant until the pod became ripe.

From the data available, it cannot be definitely established that the marked increase in mineral elements between the 87th and 107th days was significant. Two of the points show an increase, while the intermediate point (93 days) conforms more closely to the previous trend. It may be pointed out that the water content of the 93 day sample was also lower than points on either side (Fig. 1), which suggests that this sample was aberrant. It is also to be noted that this apparent increase in minerals corresponded to the period when the water content of the pod was greatest, and when the maximum increments of fresh matter and dry matter had been attained, and also to the time when most rapid changes were occurring in the bean. The liquid contents of the bean were beginning to become gelatinous, and the cotyledons beginning to grow; *i.e.* at a time when condensation of metabolites was presumably taking place and fat was forming.

If the rapid increase in the percentage ash at this stage is a real phenomenon (which can only be verified by further experiments), it is obviously of great importance in relation to cherelle wilt, for a sudden demand on the part of established pods for mineral nutrients may reduce the supply to developing cherelles, and thus cause wilting.

Table 3.

ABSOLUTE CHANGES IN ASH CONTENT OF A TYPICAL POD DURING DEVELOPMENT.

Age of pod. Days	ASH CONSTITUENTS PER POD (g).				
	Total Ash	Total N	K ₂ O	P ₂ O ₅	CaO
32	0.0286	0.0083	0.0098	0.0030	0.0051
39	0.0527	0.0152	0.0179	0.0055	0.0093
46	0.0985	0.0268	0.0343	0.0094	0.0174
52	0.1894	0.0449	0.0642	0.0172	0.0360
57	0.2355	0.0582	0.0814	0.0214	0.0446
63	0.3818	0.0942	0.1340	0.0355	0.0698
68	0.4028	0.1007	0.1359	0.0366	0.0732
73	0.5271	0.1371	0.1884	0.0520	0.0939
78	0.7372	0.1818	0.2534	0.0769	0.1323
84	0.8857	0.2291	0.3130	0.0817	0.1429
87	1.2975	0.3197	0.4795	0.1192	0.2020
93	1.5895	0.3954	0.5660	0.1505	0.2569
99	2.1008	0.5263	0.7821	0.1521	0.2969
107	2.6798	0.6973	1.0515	0.2154	0.3213
122	3.4078	0.9252	1.4186	0.3290	0.3752
143	4.5537	1.2252	1.8767	0.4533	0.4178
170	6.4899	1.5957	2.6758	0.6325	0.4670

Table 3 and Fig. 4 show the *absolute* amounts of total ash, total nitrogen, K₂O, P₂O₅ and CaO present in the pod in all stages of its history. There was a gradual increase in *total ash*, reaching a maximum between 93 and 107 days, over which period the average increment was 0.8 g. per day. From the 107th day onwards, the increment was practically linear, and the uptake of minerals did not cease when the pod ripened. The trends in *potassium* and *nitrogen* were very similar to that of total ash. In the mature pod, there was about 2.7 g. of potash. The curves for *phosphorus* and *calcium* are somewhat different from the foregoing. For approximately the first 70 days, the increases in amount of phosphorus and calcium were the same, with the ratio of CaO to P₂O₅ approximately two to one. Subsequently, the increase in uptake of phosphorus was relatively more rapid than that of calcium, and between 122 and 143 days, the absolute content of P₂O₅ became equal to the absolute content of CaO. From that point until ripening, there was more P₂O₅ in the pod than CaO. Thus, whereas half the amount of CaO present in the mature pod was attained after 90 days, half the amount of P₂O₅ present in the mature pod was reached only after about 120 days. This suggests that phosphorus is especially necessary in the later development of the pod.

(4) Summary.

1. The changes in water content, fresh weight and dry weight of the cacao pod during its development have been investigated side by side with the more obvious changes taking place in the pod.

2. The mineral intake of the pod during its development from fertilisation to maturity has also been investigated.

3. The changes in water content follow a well-marked cycle. The water content is very high in the early stages, falls, and then rises to a maximum at a time when rapid changes are occurring in the beans. Subsequently, there is a fall in water content until maturity.

4. There is a change in the growth rate at about 50 days, corresponding to the time of the first division of the zygote.

5. Potassium is the element most in demand, and the ripe pod contains about 2.7 g. of K₂O. There is a marked difference in the intake of calcium and phosphorus. Half the amount of calcium present in the mature pod is attained after 90 days, but half the amount of phosphorus present in the mature pod is accumulated only after about 120 days.

(5) Acknowledgment.

The author wishes to thank Mr. G. Rodrigues for performing the ash analyses recorded in this paper.

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III.—LEAF-FLUSH AND MINERAL INTAKE BY THE SHOOT.

By E. C. HUMPHRIES.

(1) Introduction.

PYKE (2) suggested that one of the causes of cherelle wilt might be the sudden demand made on the nutrient and water reserves of the tree by a *new flush of leaves* which results in a drain on the developing fruits. Cacao trees in Trinidad change their leaves several times a year, the frequency depending apparently on meteorological and soil factors, although the exact conditions causing leaf-change are not well understood. It seems certain, however, that trees in certain localities are more liable to leaf-flush than in other localities.

The preliminary investigation described in this paper was carried out in order to obtain knowledge of the mineral requirements of the cacao leaf-shoot during its development; also to determine the extent to which a new flush makes demands on the mineral reserves of the tree.

(2) Experimental.

SAMPLING METHODS.

For the purposes of this investigation, it was decided to take weekly samples over a period of 16 weeks. Actually, a new flush of leaves appeared nine weeks after the initial flush, (which marked the beginning of the experiment) had appeared. 50 flushes (*i.e.*, all the new growth, both stem and leaves) comprised a sample, that is, 800 flushes altogether, over a period of sixteen weeks, were collected. Aluminium labels bearing the numbers 1, 2, 3, 4, &c., up to 16 were printed, 52 of each number, and randomised between 50 trees of uniform material (Parent 1304—River Estate, already employed in the second investigation), so that each tree had 16 or 17 labels. These labels were scattered as much as possible over the tree, so that high as well as low branches were tagged. That this method of distributing the labels at random is efficient is shown by the fact that not more than four labels of any one number occurred on any one tree, and this occurred only in a few instances.

It was observed on 15th June, 1939, that a general flush on these particular trees had just commenced. (Reference to the soil moisture data in Figure 1 of Paper I of this series [Humphries (1)], shows that this was a time when the moisture of the soil was rapidly increasing.) At this stage, the young shoot was about 5 mm. long, and the young leaves were just visible. It was assumed that the shoot was one week old at this stage. 50 flushes bearing the label No. 1 were collected, and instantly placed in tins with tight-fitting lids, bound with tape, and conveyed as quickly as possible to the laboratory, where the tins were weighed and the fresh-weight of the flushes obtained. The leaves were then quickly separated from the stems and petioles, counted, and the two fractions weighed separately. These

were then dried in the oven at 105°C, and the water content calculated. The following week at the same time, 50 flushes bearing the number 2 were collected and treated in a similar manner, the last sample (No. 16) being collected on 28th September, 1939. During the ninth week, a second flush of growth was beginning on some of the shoots, and subsequently appeared on all the shoots. The leaves and stems of the new flush were separated from the original flush and weighed separately. In the fifteenth and sixteenth weeks, a third flush was beginning on many of the shoots. In the few cases where rain fell just before the sample was collected, the leaves were wiped with blotting paper before weighing.

(3) Results.

CHANGES IN WATER CONTENT OF THE STEM AND LEAF.

Fig. 1 and Table 1 show the changes taking place week by week in the water content of the stem and leaf. The water content of the newly-emerged shoot was high and rose in the second week. In the third week, the water content of the stem was only slightly higher than that of the leaf. After this, the water contents of both leaf and stem dropped markedly, the former more rapidly than the latter. The young cacao leaf is coloured with an anthocyanin pigment which masks the colour of the chlorophyll. Between the third and fourth weeks, the leaf becomes green and its surface hardens, coincident with the time of rapid decrease in water content.

Table 1.

THE CHANGES IN WATER CONTENT OF LEAF AND STEM OF A CACAO SHOOT DURING THE FIRST SIXTEEN WEEKS OF ITS DEVELOPMENT.

Age in weeks.	WATER CONTENT.	
	(Percentage Oven-dry Weight.)	
	Leaf.	Stem <i>plus</i> petiole.
1	360.60	470.32
2	423.98	524.88
3	408.50	418.38
4	242.50	364.86
5	193.07	302.02
6	195.83	291.63
7	169.38	258.30
8	180.01	282.87
9	152.84	268.19
10	154.74	255.23
11	158.48	267.60
12	153.32	263.33
13	135.56	256.84
14	133.11	229.06
15	122.13	230.39
16	124.82	237.09

Table 2.

CHANGES IN ASH COMPONENTS OF LEAF AND STEM OF A CACAO SHOOT DURING THE FIRST SIXTEEN WEEKS OF ITS DEVELOPMENT. RESULTS EXPRESSED ON THE BASIS OF DRY-WEIGHT AND ASH.

Age in weeks.	LEAF.							STEM.						
	Percent. Dry-Weight				Percent. Ash.			Percent. Dry-Weight.				Percent. Ash.		
	Ash	N	P ₂ O ₅	K ₂ O	CaO	MgO	P ₂ O ₅	Ash	N	P ₂ O ₅	K ₂ O	CaO	P ₂ O ₅	K ₂ O
1	7.66	—	1.48	2.70	0.64	0.75	19.31	12.38	—	0.65	4.20	2.07	5.24	33.93
2	7.72	3.78	1.17	2.96	0.65	0.63	15.16	13.25	—	0.62	4.24	2.32	4.68	32.00
3	6.70	3.04	0.84	2.67	0.55	0.54	12.53	12.15	1.60	0.34	3.19	2.73	2.80	26.26
4	7.05	2.22	0.59	2.14	0.62	0.45	8.37	6.40						
5	6.21	2.24	0.57	2.16	0.88	0.55	9.17	8.73	1.48	0.36	2.73	3.52	3.02	22.92
6	7.13	2.31	0.53	2.23	1.17	0.62	7.43	12.56	1.44	0.38	2.63	3.97	3.02	20.94
7	7.85	2.23	0.51	2.06	1.42	0.63	6.50	13.32	1.26	0.32	2.52	4.64	2.40	18.92
8	7.84	2.47	0.47	2.16	1.47	0.69	5.99	13.08	1.32	0.41	2.57	4.49	3.13	19.65
9	8.60	2.43	0.46	2.11	1.79	0.72	5.35	13.59	1.47	0.36	3.32	4.68	2.65	17.07
10	9.20	2.38	0.39	1.86	1.72	0.72	4.24	14.02	1.26	0.33	2.18	4.86	2.35	15.55
11	9.11	2.32	0.44	1.89	2.12	0.75	4.83	14.28	1.19	0.32	2.11	5.34	2.24	14.78
12	9.87	2.34	0.38	1.67	2.20	0.80	3.85	15.28	1.18	0.33	2.02	5.89	2.16	13.22
13	10.27	2.26	0.35	1.47	2.62	0.80	3.41	14.50	1.10	0.27	1.86	5.72	1.86	12.83
14	11.40	2.32	0.32	1.41	2.93	0.79	2.81	15.73	1.07	0.25	2.02	6.42	1.59	12.84
15	10.96	2.01	0.34	1.36	2.76	0.80	3.09	14.74	1.02	0.22	1.65	6.07	1.49	11.19
16	11.81	2.00	0.32	1.28	3.00	0.89	2.71	15.30	1.07	0.20	1.78	6.12	1.31	11.63

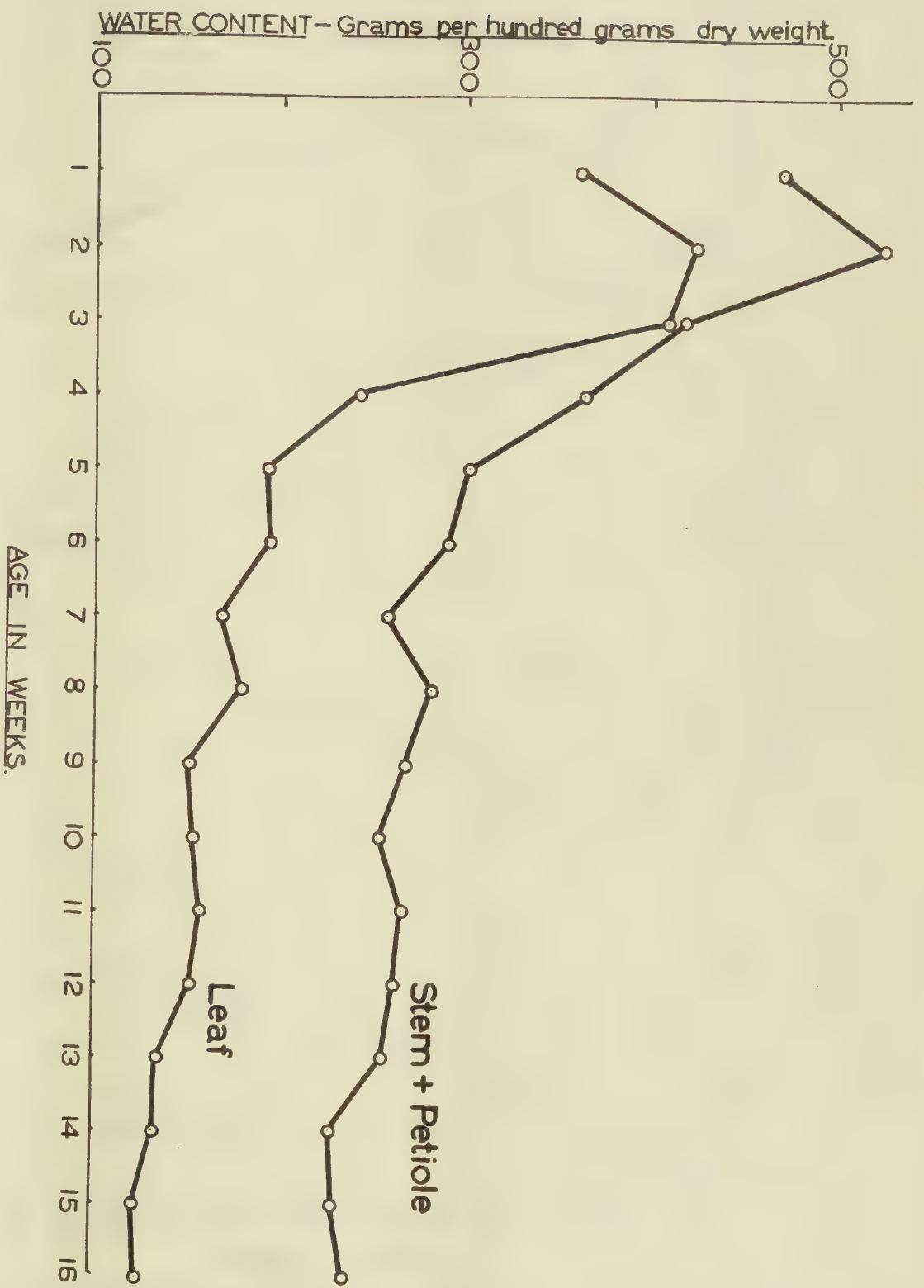


Fig. 1. The changes in water content of the leaves and stem of a cacao shoot for a period of sixteen weeks after the bud opens.

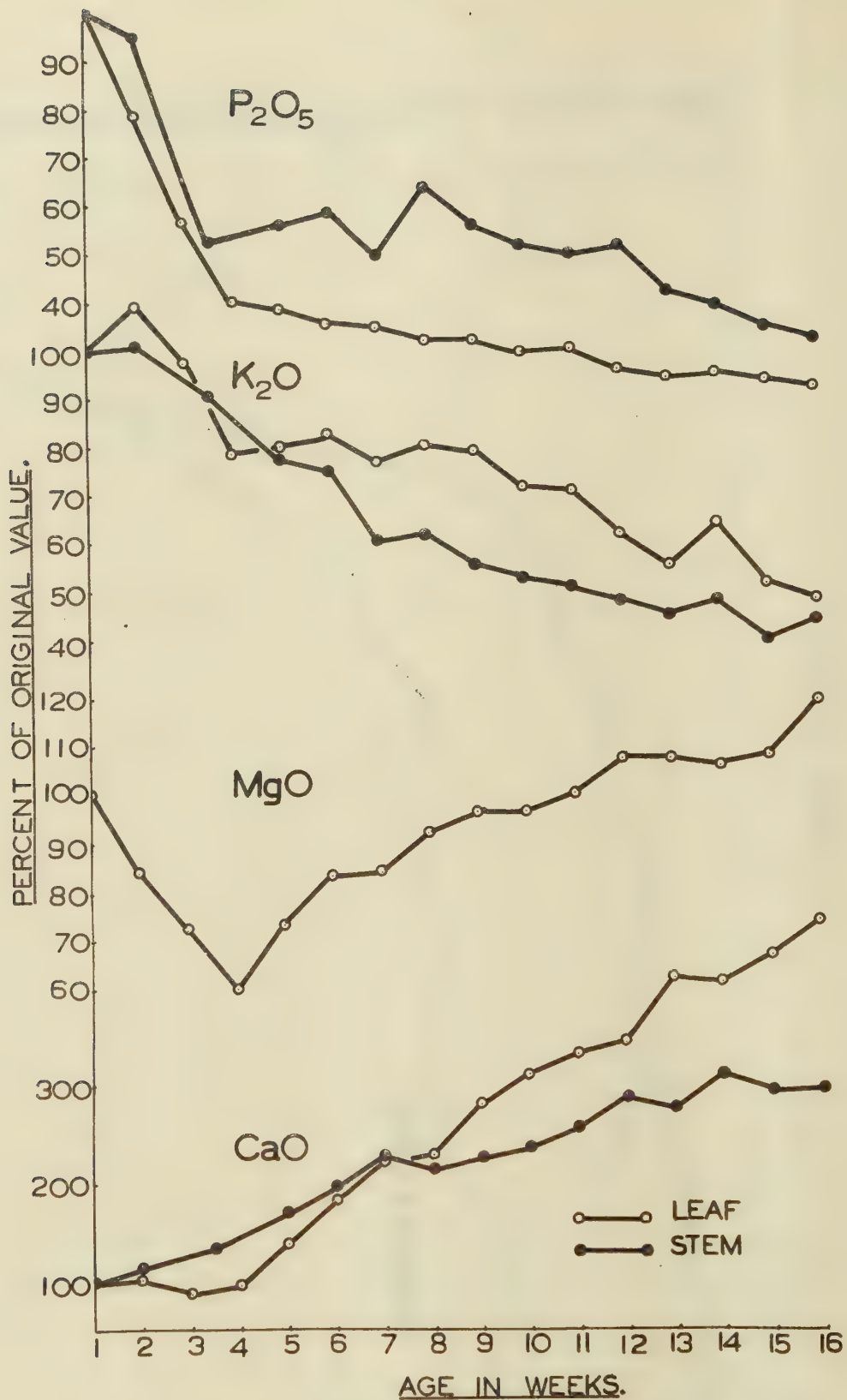


Fig. 2. The drift of certain ash constituents of the leaf expressed as percentage of the value during the first week. The results are calculated on a dry weight basis.

From this time onwards, there was a gradual decrease in water content, both in the stem and in the leaf. At the sixth week, the water content of the leaf was about 200 g. per 100 g. dry matter and that of the stem, 300 g. per 100 g. dry matter. At the tenth week, the leaf contained 150 g., and the stem 250 g. of water, per 100 g. of dry matter. At the sixteenth week, the figures were 120 g. and 240 g. respectively. At this stage, the leaves were extremely brittle and some showed signs of withering. It will be noticed from Fig. 1 that there was a parallel march of changes in water content in the leaf and stem.

CHANGES IN ASH COMPONENTS OF THE LEAF AND STEM.

The analyses of the ash of stem and leaf at different ages are set out in Table 2. The results are expressed as percentages of dry-weight and as percentages of ash. There was a general decrease in the percentage of total ash until about the fourth week, when an increase commenced and was maintained. The changes which took place in the ash components are more clearly seen when the results are expressed as percentages of the original value (*i.e.* the value found for the first sample). The calculated results for P_2O_5 , K_2O , CaO and MgO are plotted in Fig. 2.

Changes in the leaf.

In the leaf, there was a definite change at the fourth week in all the components; *i.e.*, at the time when the leaf hardens. After four weeks, the phosphorus content had fallen to 40 percent. of its original value (expressed as percentage of the dry-weight), but, during the succeeding 12 weeks, the value only fell about another ten percent. The percentage potassium in the dry matter of the leaf rose in the second week, and then fell to about 80 percent. of the original value in the fourth week. It then fluctuated about this mean value until the ninth week, when a decline commenced which continued for the rest of the time. The changes taking place in magnesium content were very marked. There was a steady decline during the first four weeks to 60 percent. of the original value, after which an increase took place. At the ninth week, the value was about equal to the original value, and the rise continued for the rest of the time of the investigation. At the sixteenth week, it was about 120 percent. of the original value. Calcium showed very little change during the first four weeks, but subsequently there was a rapid rise, the final value (sixteenth week) being 470 percent. of the original value.

The fall of phosphorus content of the leaf, expressed on a dry-weight basis, follows very closely the changes in water content, which suggests that the amount of phosphorus, expressed in terms of water, will be sensibly constant in successive weeks. This is actually

found to be the case. The following are the calculated values of mg. of P_2O_5 per 100 g. of water in successive weeks:—

4.1, 2.8, 2.1, 2.4, 3.0, 2.7, 3.0, 2.6,
3.0, 2.8, 2.8, 2.5, 2.6, 2.4, 2.8, 2.6.

This relationship might prove to be of importance in investigations of nutritional status of the cacao tree growing in a particular environment.

Changes in the stem.

A decrease in percentage of phosphorus occurred until between the third and fourth weeks, when a slight increase took place, until the ninth week, after which it diminished. In the case of potassium, except for a very slight increase in the second week, there was a steady decline throughout the whole period. Calcium showed an increase throughout, reaching a figure of 300 percent. of the original value on the sixteenth week.

ABSOLUTE CHANGES PER FLUSH.

Since the dry-weight changes taking place in the leaves and stem of the flush throughout its development are known, it is possible to calculate the *absolute* changes in the different ash components in successive weeks.

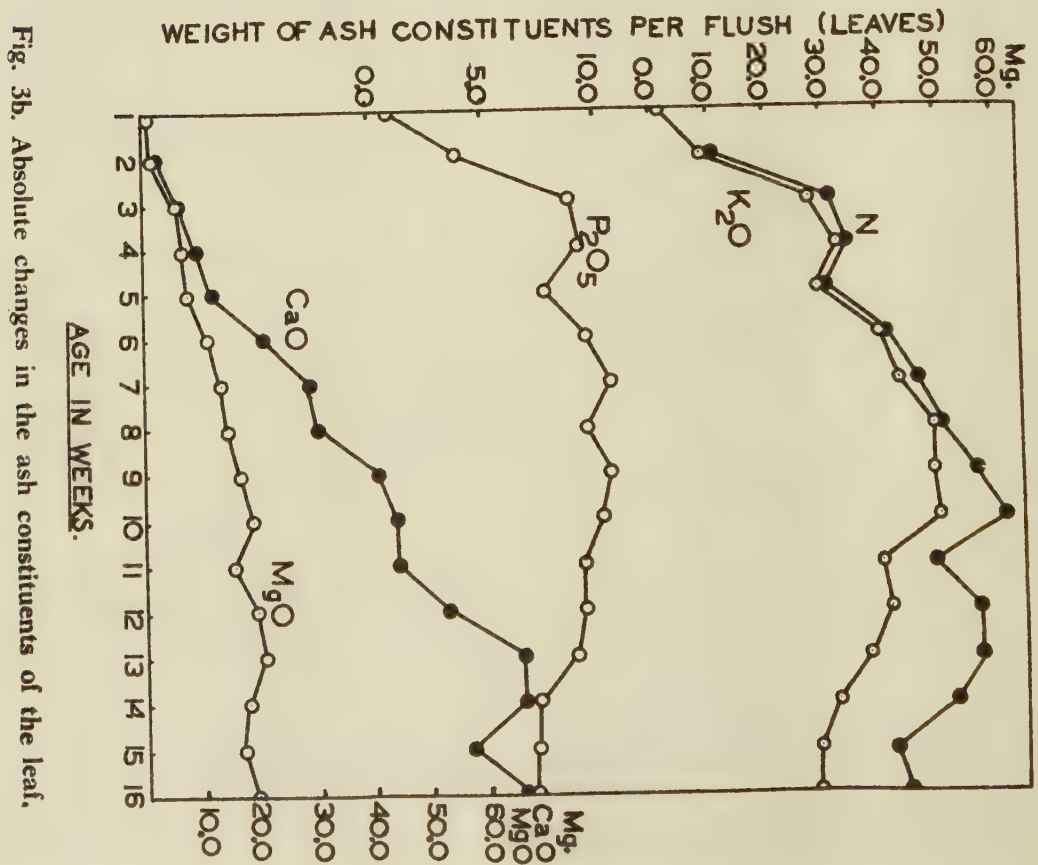
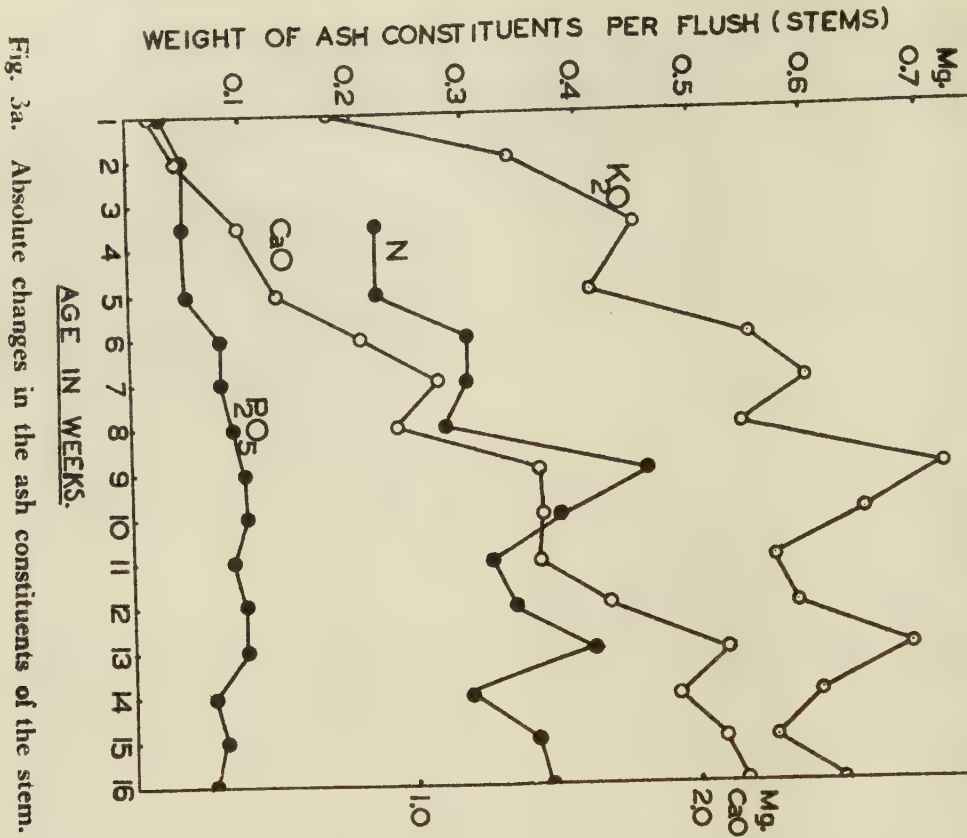
Stem.

The absolute changes in content of potassium, nitrogen, calcium and phosphorus of a single stem are shown in Fig. 3A. In all cases, there was an increase until the ninth week, after which the potassium content appeared to fluctuate around a mean value. There appeared to be a small decrease in nitrogen and phosphorus, indicating that a slight transport out of the stem took place. Calcium showed an increase over the whole period.

Leaf.

The absolute changes taking place in certain components of the leaves of a single flush are plotted in Fig. 3B. In the case of nitrogen and potassium, there was an increase up to the tenth week, after which there was a progressive decrease. That is, at the tenth week, transport of nitrogen and potassium out of the leaf apparently began to take place, and was continued for the rest of the time. In the case of phosphorus, there was a similar decline, which, however, began after the ninth week.

There can be no doubt that the loss of minerals from the leaf was due to the advent of the second flush, which began in the ninth week. This immediately affected the phosphorus content of the leaf, and transport took place immediately. In the case of nitrogen and potassium, the effect was felt in the following week. Thus, in the sixteenth week, the content of nitrogen fell to 70 percent. of its peak value of the tenth week, potassium to 56 percent., and phosphorus to 66 percent. In the case of calcium and magnesium, no transport out of the leaf could be detected.



There was no indication that any recovery of minerals took place in the old leaves, and the net loss of minerals which took place through the advent of the second flush, must have been an important factor in hastening senility of the leaves with consequent loss of efficiency, finally leading to leaf-shedding. Thus, in the particular instance studied, the average life of the leaves of the flush was just over sixteen weeks, which seems extremely short. The question arises as to whether, if more mineral nutrients were available, the old leaves would be able to make up the mineral deficiencies occasioned by the growth of a new flush, and would consequently retain their efficiency for a longer period of time. Casual observation suggests that, on richer soils, cacao leaves tend to remain healthy for a longer time than was the case at the site under observation. It is hoped later to study the changes in composition of leaf-flushes for cacao trees growing in other environments in which the soil has different nutrient status from that occurring at River Estate.

(4) Summary.

1. The changes in mineral content of the cacao shoot during development have been investigated by means of chemical analyses of weekly samples.

2. The onset of a new flush, nine weeks after the experiment had commenced, caused a transport of potassium, nitrogen and phosphorus out of the leaf, which loss was not made good by the tree. It is suggested accordingly that this failure may be one cause of premature senility of the leaves at this site.

3. The phosphorus content of the leaf, expressed on the basis of 100 g. of water, remained practically constant over the period investigated.

(5) Acknowledgment.

The author wishes to thank Mr. G. Rodrigues for performing the ash analyses recorded in this paper.

(6) References.

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PART III.—BIOCHEMISTRY OF CACAO.

INVESTIGATION OF THE PURPLE COLOURING MATTER OF CACAO BEAN.

BY H. F. BIRCH.

(I) Introductory.

Of the various substances of the cacao bean that undergo alteration during fermentation, the tannin, catechin, fat and alkaloid components have already been investigated, and the results described in the last three Annual Reports. The changes which these substances undergo may be brought about either by physical processes, such as diffusion, or by chemical transmutations. It is these chemical changes that are presumed to be mainly responsible for the superiority of fermented over unfermented cacao. In the coloured beans of Forastero and related cacao varieties, there is present a pigment which has been partly identified as cyanidin-3-glycoside, though not yet isolated in the pure state. During fermentation, there is a marked loss of pigment, a change which may be greatly advantageous, since the pigment is stated to possess a harsh astringent taste, and its occurrence in prepared cacao might therefore lower the value of the product. That the presence of anthocyanin pigment partly determines the quality of cacao, is supported by the fact that Criollo cacao (a colourless variety) is superior to the pigmented types. From recent evidence, however, it appears

that there is present in Criollo cacao a leuco-anthocyanin, which is a colourless compound closely related to the cyanidin-3-glycoside found in Forastero cacao. It is reasonable to suppose that this leuco-anthocyanin confers considerably less harsh characteristics on cacao bean than the true pigment, or that it undergoes more rapid chemical change during fermentation. With Criollo cacao, a two-day fermentation is usually practised and apparently is all that is needed, whereas with Forastero cacao, an eight to ten-day fermentation is required. Since the content of tannin (which also undergoes change during fermentation) has been proved to be approximately the same in the two varieties, the difference in time necessary for their fermentation may now be attributed to the presence of purple pigment in the one case and its absence in the other.

(II) Some Preliminary Investigations.

The biochemical investigations at the College were first directed to the isolation of a pure specimen of the pigment from Forastero cacao. The object of this was two-fold, firstly to confirm the nature of the pigment, and secondly to trace the rate at which it disappears during fermentation.

It was considered by the author that, given (i) a series of standard solutions of the pure pigment, (ii) an electrophotometer, and (iii) coloured extracts prepared from cacao at different stages of controlled fermentation, it should be possible to follow the rate of disappearance of the pigment, and perhaps to establish a correlation between pigment content of well-fermented and poorly-fermented cacao and the time required for fermentation. Since the leuco-anthocyanin in Criollo cacao can easily be converted in the laboratory to the coloured analogue, the same technique could be applied in order to follow the rate of disappearance of leuco-anthocyanin in Criollo cacao during its fermentation.

Experimental.

The isolation of the sugar-free pigment, cyanidin, from the cyanidin-3-glycoside present in Forastero cacao, was first attempted. This substance might be used as standard for following the pigment changes in fermenting cacao, by hydrolysing the pigmented extracts of the cacao at different stages of fermentation so as to obtain the simple cyanidin in solution. Two kilograms of fresh, shelled, stabilised and finely-minced cacao kernels were extracted with dilute hydrochloric acid, and the reddish-purple coloured solution was hydrolysed to convert the glycoside to cyanidin. It was noticed that hydrolysis was accompanied by the deposition of a considerable amount of a dark-brown insoluble product. After separating the insoluble matter from the solution, this was then extracted several times with amyl alcohol, and the characteristic intense purple solution of cyanidin in amyl alcohol was so obtained. Repeated attempts to prepare a pure specimen of cyanidin from this solution by methods of fractional precipitation and crystallisation unfortunately were unsuccessful, nor was it possible to obtain a specimen of cyanidin from the insoluble material deposited during hydrolysis of the glycoside. Judging by the small quantity of impure cyanidin obtained, it appears that the cyanidin is precipitated together with a considerable amount of a substance or substances having properties similar to it, and that, during the long precipitation process necessary for the separation of the cyanidin from these associated substances, it undergoes oxidation or condensation, with

the formation of brown polymers. This possibility is supported by the fact that, whereas the initial solution of the pigment in amyl alcohol definitely showed all the reactions of cyanidin, the crude product precipitated from the solution showed them only to a small extent, and the final product, obtained after a long process of purification, hardly at all.

An attempt was next made to isolate the pure pigment, cyanidin-3-glycoside, from Forastero cacao. Extraction of the glycoside with glacial acetic acid, followed by precipitation with ether, proved unsatisfactory, on account of the considerable amount of other substances also precipitated. Another method of approach, namely, the precipitation of the anthocyanin as a salt from a methyl alcohol hydrochloric acid extract of Forastero cacao, was also tried, but this too proved unsuccessful. Here again, many other compounds having properties closely related to those of the anthocyanin were simultaneously precipitated, and the separation of the colouring matter from them was impossible, doubtless because of the unstable nature of the pigment.

Since a pure specimen of the pigment could not be isolated, recourse might in future be made to a comparison of the intensity of colouration in pure solutions of the pigment prepared by extraction of cacao at different stages of fermentation under identical conditions. In this way, it ought to be possible to determine the *relative* amount of anthocyanin present at any particular stage of fermentation, the rate at which it undergoes change, and the stage at which it is present in least amount.

(III) Summary.

(1) Attempts to isolate cyanidin from the purple pigment (cyanidin-3-glycoside) of Forastero cacao bean were unsuccessful, owing to its very unstable nature.

(2) Attempts to isolate the glycoside were likewise unsuccessful, as was also an attempt to isolate the hydrochloride, for the same reason.

(3) It is suggested, that in order to trace the rate of decomposition and loss of purple pigment during fermentation, and to compare in this respect Forastero with Criollo cacao, colour-comparison of extracts, prepared under standard conditions, might prove successful.

